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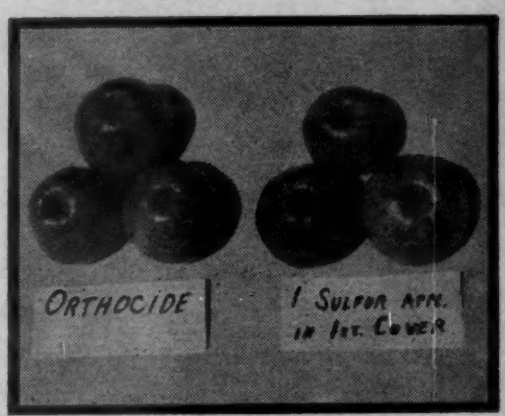

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An International Journal

Official Organ of The American Phytopathological Society

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CLARENCE MITCHELL TUCKER, 1897-1954

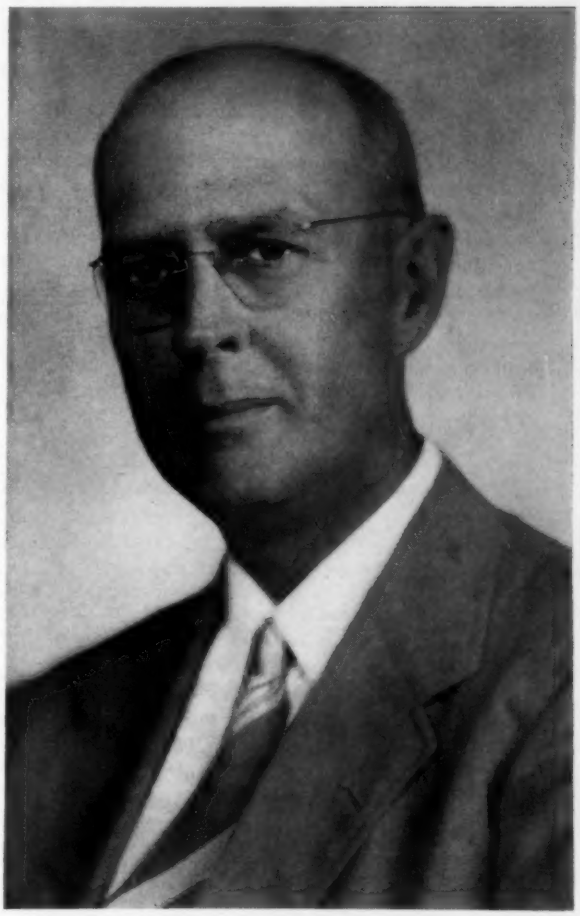
T. W. Bretz

Clarence Mitchell Tucker, Professor and Chairman of the Department of Botany at the University of Missouri, died after a prolonged illness on February 3, 1954, in Columbia, Missouri.

The son of Joseph Davis and Lena Theophilus (Swinney) Tucker, Mitchell was born in Centralia, Missouri, on October 28, 1897. He received the baccalaureate degree in Agriculture from the University of Missouri in 1920, after having served with the U. S. Marine Corps in 1918-19. He became Professor of Plant Pathology at the University of Puerto Rico in 1920, and in 1923 was appointed Plant Pathologist in the Federal Agricultural Experiment Station, Mayaguez, Puerto Rico, a position he held until 1930. He spent the academic years 1926-27 and 1929-30 in research and graduate study at the University of Missouri and the Boyce Thompson Institute. In 1930 the University of Missouri awarded him the degree, Doctor of Philosophy. Dr. Tucker served as Associate Plant Pathologist in the Florida Agricultural Experiment Station the following year, and returned to the University of Missouri as Associate Professor of Botany in 1931. In 1937 he was appointed Professor and in 1938 became Chairman of the Botany Department, in which capacity he served until the time of his death.

As an outgrowth of work on coconut bud rot, caused by *Phytophthora palmivora* Butler, during his first years in Puerto Rico, Professor Tucker became interested in the taxonomy of the genus *Phytophthora* de Bary. Through his researches he acquired an international reputation as an authority on this group of fungi, and he maintained his eminence in this field throughout his career. He will be best remembered in Missouri for his work on the development of tomato varieties highly resistant to Fusarium wilt that yielded exceptionally large harvests when grown under typical Missouri field conditions. His active research in this and other phases of plant pathology were recorded in a continuous series of publications, and attracted a growing group of advanced students who profited much from his warm personal interest and broad technical knowledge.

Through his quiet dignity and modesty, Mitchell Tucker's high motives and incisive mind were ever apparent. Appointments and elective offices that required objective judgment and trust gravitated to him. He served as Councilor-at-Large in the American Phytopathological Society during 1946-47, as its Vice President in 1949 and its President in 1950. During 1946-48 he was an Associate Editor of Phytopathology. He held several key administrative posts in the University of Missouri including the chairmanships of the University Policy Committee, the Wildlife Council, and Arts and Science Personnel Committee. During the academic year 1951-52 he served as Acting Dean of the College of Arts and Sciences. His University colleagues elected him President of their chapter of Sigma Xi, President of the University Club, and Arts and Science Representative on the University Committee on Tenure. He held memberships in the American Society of Botany, the Mycological Society of America, the American Association for the Advancement of Science, the Missouri Academy of Science, the Missouri Historical Society, Sigma Phi Epsilon, and Rotary International.



CLARENCE MITCHELL TUCKER
1897-1954

On September 15, 1920, he married Helen Baskwell Jones of Ocala, Florida. Besides his wife he is survived by their only child, Jacqueline Lee (Mrs. James C. Dowdy), one brother and two grandchildren.

The memory of Professor Tucker's inspiring scientific leadership, his sense of fairness, and his insight into human values will remain an enduring influence at the University of Missouri and among his colleagues in the field of plant pathology.

PHYTOPATHOLOGY for June (45: 295-350) was issued June 10, 1955.

STUDIES ON STEMPHYLIUM LEAFSPOT OF ALFALFA[1]

R. R. Nelson

SUMMARY

Stemphylium leafspot of alfalfa is an important problem in Minnesota. The fungus overwinters principally as immature perithecia on plant debris and in dormant plant tissues. Symptoms from ascospore infections appear in early spring and the infection cycle of ascospores to ascospores is repeated several times until early July, then the pathogen is perpetuated only by the conidial stage.

The pathogen infects leaflets, stems, petioles, peduncles, pods, and seeds of alfalfa, causing defoliation, wilting, and dwarfing. Entire stems are killed and seeds can become infected.

In the greenhouse approximately 70 per cent of the infected leaflets dropped from the plants. The amount of defoliation is determined directly by the position and number of lesions, petiole infection, size and position of lesions, and age of plants.

Stemphylium leafspot depresses both yield and quality of alfalfa seed. Losses in yield are caused directly by fewer pods produced, fewer seeds per pod, and lighter seed. Quality is affected by a lower germination percentage of infected seed and by the production of dark-colored shriveled seed.

The potential importance of Stemphylium leafspot during any given year is dependent largely upon moisture and temperature. The optimum conditions for initial infection are temperatures of 18–24°C with 100 per cent relative humidity for at least 12 hours. Temperature has more effect than moisture on the further development of the disease. Moderate temperatures are most conducive for disease development.

The widespread occurrence of leafspot diseases greatly affects the yield and the quality of forage and seed of alfalfa in Minnesota. Stemphylium leafspot, caused by *Stemphylium botryosum* Wallroth, is epidemic in localized areas in most years and frequently becomes epidemic over wide areas. The present studies were undertaken to learn more about the pathological aspects of the disease and its causal agent.

Although *Stemphylium botryosum* is generally considered to be the imperfect stage of *Pleospora herbarum* Rabehn. (3, 6, 7, 8), evidence gained in the present studies indicates that it probably is connected also with *Pseudoplea briosiana* (Pollacci) von Höhnel (4). The present account deals with the more strictly pathological aspects of the problem. Since the causal agent conforms to the description of *S. botryosum* it will be referred to as *S. botryosum* and the disease will be referred to as Stemphylium leafspot.

MATERIALS AND METHODS.—One hundred isolates of *Stemphylium botryosum* and 65 isolates of *Pseudoplea briosiana* were obtained from widely scattered areas in Minnesota. Five isolates of *S. botryosum* from red clover were used for cross-inoculation studies on red clover and alfalfa and for a morphological comparison with isolates from alfalfa.

Stemphylium isolates were obtained by surface disinfesting bits of infected leaf tissue and plating on potato-dextrose agar acidified with lactic acid. When cultures had grown sufficiently for positive identification, transfers were made to agar slants in tubes. Monoconidial isolates were obtained from each mass isolate. Spore suspensions from the mass conidial cultures were atomized onto agar plates and single germinating conidia were isolated with a modified Keitt "biscuit cutter" (2) and transferred to test tubes.

Single ascospore isolates of *P. briosiana* were obtained by taping infected leaflets to the inside of the cover of a Petri plate, so that ascospores could be ejected onto the agar. Germinating ascospores were isolated singly with the techniques described for isolation of conidia.

In the field, alfalfa plants were inoculated with a suspension of conidia in water applied with either a knapsack sprayer or a power sprayer, the latter at 110 lb. pressure. Inoculum grown on liquid or agar media was comminuted in a Waring Blendor, diluted with water, and added directly to the sprayer. Inoculum grown on grain or alfalfa media was mixed thoroughly in water and strained into the sprayer. Inoculum was obtained also from infected leaflets which had been placed in a moist chamber to induce sporulation, and then soaked in water and strained into the sprayer. Similar techniques were used to inoculate plants in the greenhouse.

The relative humidity in the field was increased by building plastic tents over the areas in which plants to be inoculated were growing. Plants inside the tents were sprayed with water several hours prior to and after inoculation until the first symptoms appeared. In the greenhouse, plants were incubated at 100 per cent relative humidity in plastic moist cham-

[1] Accepted for publication February 9, 1955.

Part of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology. The author is grateful to Dr. M. F. Kernkamp for his helpful suggestions throughout the course of this investigation.

Paper No. 3299, Scientific Journal Series, University of Minnesota, Agricultural Experiment Station.

bers. Plants were kept at 18°C during and following incubation unless otherwise stated.

DESCRIPTION OF THE DISEASE.—Symptoms of Stemphylium leafspot have been described previously by Smith (6) and Benedict (1). The observations made during the present studies agree, for the most part, with the previous descriptions.

Stemphylium leafspot attacks all of the aboveground parts of alfalfa, including leaves, stems, petioles, peduncles, flowers, and pods.

Symptoms from ascospore and conidial infections first appear on alfalfa leaflets as punctate lesions, 1-2 mm in diameter, with light-colored centers and reddish brown margins (Fig. 1, A). After several days, lesions from ascospore infections become large and somewhat irregular in shape. The reddish margin is usually a distinct part of the growing lesion, but frequently becomes less defined as the lesion attains a diameter of several mm. Larger lesions have grayish tan centers and contain numerous perithecia (Fig. 1, B).

In early summer, conidia are produced in lesions that result from ascospore infections. These olive brown to dark brown lesions usually are similar in shape and size to those containing only perithecia (Fig. 1, C). Older lesions from conidial infections become large, irregular, and dark brown and are accompanied by increasing necrosis and chlorosis of the leaflets. During moist periods these lesions become blackened by tremendous numbers of conidia and the leaves appear watersoaked. Lesions commonly coalesce to form large diseased areas and concentric rings often develop in the older lesions (Fig. 1, D).

Black lesions on stems and petioles appear in early summer. During wet seasons these lesions elongate, coalesce, and frequently girdle entire stems and petioles causing their death.

Later in the growing season, the pathogen attacks the flowers and subsequently infects the seeds. Infected seeds occasionally are shriveled and dark colored, but more often the symptoms are less obvious. Deformed pods typically result from floral infections.

Pronounced wilting and stunting of the plants usually accompany severe infections. Stunting is especially evident if the plants are infected when young, either in the spring or following cutting. Plants severely infected usually are chlorotic and the noninfected leaflets wilt (Fig. 1, E).

LIFE CYCLE OF THE PATHOGEN.—The fungus overwinters as immature perithecia on plant debris and in dormant plant tissues. Ascospores constitute the major source of primary inoculum, although conidia occasionally are produced early in the spring. About 2 weeks after the initial symptoms from ascospore infections appear in the spring, a new crop of perithecia is produced in the lesions, and the newly formed ascospores reinfect alfalfa leaflets. This infection cycle of ascospores to ascospores is repeated several times from early May through June. In late June, ascospores and conidia are produced in the same lesions, but by mid-July only conidia are formed in the lesions. From mid-July throughout the summer and into the fall, the pathogen is perpetuated almost exclusively by conidia. In late fall, perithecial initials form in the lesions on mature plants and on plant debris, and the life cycle is repeated.

HISTOLOGICAL STUDIES.—Studies were made on the penetration of the host by infection hyphae. Alfalfa plants were inoculated with conidial suspensions and incubated in a moist chamber for 24 hours. Inoculated leaves were cleared in a solution of 3 parts 95 per cent alcohol to 2 parts acetic acid. The cleared leaves were stained for 24 hours in cotton blue, then stained for 48 hours in a mixture of cotton blue and lactophenol, and finally fixed in pure lactophenol for 48 hours.

Infection hyphae usually enter through stomata but

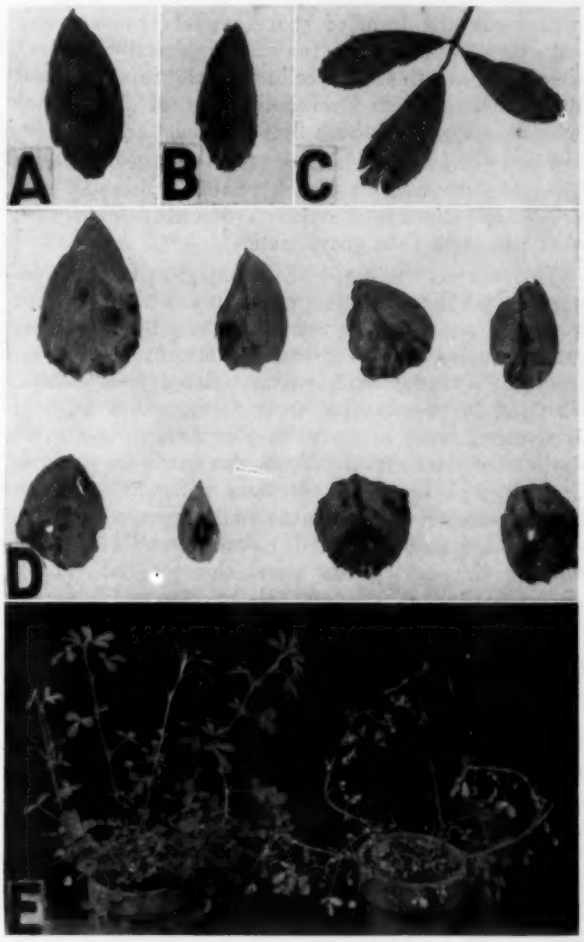


FIG. 1. Symptoms of Stemphylium leafspot of alfalfa. A) Initial symptoms of conidial and ascospore infections. B) Development of lesions with only perithecia. C) Lesions from ascospore infections containing only conidia. D) Lesions produced by the conidial stage during the summer after the perfect stage is no longer present. E) Wilting and stunting of alfalfa by Stemphylium leafspot. Left: noninoculated plant. Right: inoculated plant.

occasionally the hyphae penetrate the epidermis directly. When the germ tube enters through a stoma it retains its original size, 2–3 μ, but when it penetrates the epidermis directly the mycelium enlarges to 5–6 μ above the point of entrance.

For a more detailed study of the fungus after penetration, sections of leaf tissue containing punctate lesions were killed and fixed in Belling's modified Navashin fluid (5), dehydrated in the normal butyl alcohol series and imbedded in paraffin. Microtome sections, 8μ and 10μ thick, were stained with the modified quadruple stain (5).

A dense cluster of hyphae develops in the substomatal cavity. Many of these hyphae apparently develop no further, while others grow out from the substomatal region and ramify between the host cells. Occasionally a new area, 5–7 mm from the initial infection point, develops where the mycelium again clusters in the form of short hyphal branches and subsequently behaves in the manner described. Dead areas that are free of mycelium develop up to several mm in front of and on both sides of the hyphal strands, suggesting action by a toxin.

Upon direct penetration, the enlarged mycelia cluster in the form of short hyphal branches and subsequently behave in the manner described for hyphae that penetrate through stomata.

Host range studies.—No attempt was made to determine the complete host range of the pathogen, but plants of several of the more important forage species were inoculated with cultures of *Stemphylium botryosum* and *Pseudoplea briosiana* isolated from alfalfa. Conidial isolates derived from Stemphylium infected a greater number of forage species than did ascospore isolates derived from Pseudoplea and the isolates from Stemphylium were the more pathogenic ones on forage species susceptible to both types of isolates. Alfalfa (*Medicago sativa* L.), burr clover (*Medicago hispida* Gaertn.), yellow sweet clover (*Melilotus officinalis* (L.) Desr.), and alsike clover (*Trifolium hybridum* L.) were highly susceptible to the conidial isolates, while conidial infections on red clover (*Trifolium pratense* L.), Ladino clover (*Trifolium repens* L.), and white clover (*Trifolium repens* L.) were restricted to small marginal lesions and minute flecks. Birdsfoot trefoil (*Lotus corniculatus* L.) was the only forage species not infected by the conidial isolates. The isolates derived from Pseudoplea infected only alfalfa, burr clover, and yellow sweet clover. This is the first time that sweet clover has been identified as a susceptible host of *P. briosiana*. The difference in the host range between the isolates derived from Stemphylium and those derived from Pseudoplea may be due to differences in physiological races.

Defoliation studies.—It is generally believed that leafspot diseases cause defoliation of alfalfa. However, there are no specific data on the amount of damage done, nor has any attempt been made to evaluate the factors that influence the degree of defoliation.

Wind, rain, insects, and other factors undoubtedly aid in the defoliation of alfalfa plants in the field. Experiments were made in the greenhouse, therefore, in an attempt to eliminate or minimize as many of the variable factors of the environment as possible. Under such conditions, the amount of defoliation of inoculated plants would not be a true estimate of the effect of the disease under field conditions, but probably would be an indication of the least amount of damage done. Under controlled conditions, however, a more precise evaluation could be made of the parasitic action of the fungus.

Four factors were considered in determining the direct effect of the pathogen. They were 1) petiole infection, 2) number of lesions on a leaflet, 3) position of the lesions, and 4) size of the lesions. The last named factor was evaluated alone and also in relation to lesion position. To analyze the effect of the position of lesions, a leaflet was divided arbitrarily into 3 regions as follows: 1) basal, the third of the leaflet nearest the petiole; 2) middle, the middle third of the leaflet; and 3) apical, the terminal third. The positions of the lesions were defined further as marginal and nonmarginal, a marginal lesion being any lesion that reached the margin of the leaflet before the lesion was 2 mm in diameter.

Ten days after inoculation the numbers and positions of the lesions on individual leaflets were recorded. These data were analyzed in relation to the degree of subsequent defoliation. Leaflets on the check plants had no lesions. In some cases, the petioles of check plants were infected; these served as checks against those having both petiole and leaflet infections.

When the notes on infection had been recorded, a numbered tag on a silk thread was attached to the petiole of each leaflet. Only 1 leaflet on a petiole was studied. Marked leaflets were observed daily and the dates recorded when the leaflets dropped. Lesion size was measured at regular intervals.

Sixty-seven per cent of the 438 infected leaflets observed dropped from the plants in less than 4 weeks. Leaf-drop was greater from infected petioles than from noninfected ones (Table 1). Infected petioles and their leaflets sometimes were lost, even when all of the leaflets were without lesions. From both infected and noninfected petioles, the extent of leaf-drop was correlated with the number of lesions per leaflet. The amount of defoliation was correlated with the position of the lesions (Table 2). Leaflet-drop was most prevalent when lesions were located at the base of the leaflets. The leaflets that did not drop had very few basal lesions. The effect of position of the lesions was similar with leaflets on noninfected petioles. When leaflets had 8 or more lesions, defoliation was so heavy that an evaluation of position effect could not be made. When such data were included, the data show that of the 331 lesions on the 142 leaflets that remained on the plants, 33 were basal, 117 were

TABLE 1.—*Defoliation data tabulated according to the number of Stemphylium leafspot lesions per alfalfa leaflet and to whether or not the petioles were infected*

No. lesions per leaflet	Proportion of leaflets that dropped[a]	
	Petioles infected	Petioles not infected
0	4/38[b]	0/38
1	44/65	20/62
2	26/32	18/38
3	18/28	16/30
4	16/20	11/17
5	16/18	8/12
6	12/13	8/12
7	14/14	9/12
8	4/4	8/13
9 or more	14/14	34/36
All leaflets	164/206	132/232

[a] Denominator is number of leaflets dropped and the numerator the number observed.
[b] Four leaflets dropped because the petiole dropped.

TABLE 2.—*Defoliation data tabulated according to the number and position of Stemphylium leafspot lesions on alfalfa leaflets*

Lesions per leaflet	Number leaflets	Total number of lesions	Number of lesions at positions[a]		
			B	M	A
	On leaflets that dropped				
0	4	0	0	0	0
1	64	64	47	4	13
2	44	88	60	8	20
3	34	102	59	10	33
4	27	108	58	16	34
	On leaflets that did not drop				
0	68	0	0	0	0
1	61	61	4	38	19
2	26	52	3	34	15
3	24	72	9	30	33
4	10	40	2	30	8

[a] B, M, and A refer to basal, middle, and apical regions of the leaflet, respectively.

apical, and 181 were middle. Of the 1398 lesions on the 296 leaflets that dropped, 720 were basal, 235 were apical, and 443 were middle.

Approximately 65 per cent of the 1739 lesions recorded were marginal lesions. The marginal and nonmarginal lesions differed significantly in rate of growth and in maximum size. Nonmarginal lesions seldom attained a diameter of more than 3 mm and often remained in the red-ring stage. Marginal lesions developed to considerable size in a few days. Marginal lesions in the basal area were the most damaging lesions on the leaflets. Eighty-five per cent of the leaflets that had 5 or more lesions dropped. Nearly all of the lesions on leaflets that did not drop were in the middle third region or were nonmarginal. Lesions ranged from 1 to 10 mm in length or diameter. No leaflet with a lesion 5 mm or larger remained on the plant.

Further defoliation studies were made with plants in the flowering stage. Leaflets and petioles on older plants showed severe symptoms in 6-8 days, whereas the lesions on leaflets of younger plants still would be in the red-ring stage and few, if any, lesions would have appeared on the petioles in this length of time.

In this study, nearly 80 per cent of 400 leaflets dropped in less than 20 days. The greater susceptibility of older plants made it difficult to determine the relative importance and interaction of petiole infections, position of the lesions, and size and numbers of lesions. Only 56 of the 400 leaflets remained on the plants. None of the lesions were basal and none of the leaflets had more than 3 distinct lesions. Relatively few distinct lesions were produced because complete chlorosis and death of the leaflets occurred in a few days and coalescing of the lesions was common.

EFFECT OF STEMPHYLIUM LEAFSPOT ON SEED PRODUCTION.—During the present studies, *Stemphylium botryosum* was isolated frequently from naturally-infected seed that ranged in appearance from normal shape, size, and color to black and shriveled.

Alfalfa plants in the greenhouse were hand-pollinated and inoculated with a conidial suspension of *S. botryosum*. The plants were incubated 48 hours and kept at 19°C for 10 weeks. The mature pods then were picked and the seed threshed by hand. Noninoculated check plants were sprayed with water and otherwise treated in the same manner.

Stemphylium leafspot reduced the number of pods per plant and the number of seeds per pod. Flowers on severely infected peduncles produced aborted pods and sometimes no pods at all. Pods on infected plants frequently turned brown and appeared mature 3 weeks before pods on healthy plants were ready for harvest. Pods from infected plants had only 3-4 seeds, whereas pods from healthy plants had 5-7 seeds.

Seed from infected plants of all clones and varieties tested weighed no more than ½ and often less than 1/3 as much as seed from noninfected plants of the same clones and varieties (Table 3). Individual seeds were weighed to the nearest 0.1 mg.

The germination of seeds from inoculated plants ranged from 41 to 60 per cent and of seeds from noninoculated plants from 68 to 80 per cent (Table 3). None of the seeds from the check plants was infected, whereas up to 30 per cent of the seeds from inoculated plants were infected.

PARASITIC RACES.—Wisconsin Mildew Resistant red clover and Grimm alfalfa plants were inoculated with isolates of *S. botryosum* from alfalfa. All of the isolates produced similar large lesions on alfalfa and each produced the perfect stage, *Pseudoplea briosiana*, on mature alfalfa plants. Some of the isolates produced large marginal lesions on red clover, but others caused only numerous flecks that developed no further. Red clover was resistant to some of the isolates tested. Only the conidial stage was reisolated from the lesions on red clover.

Differences in pathogenicity among isolates of *S.*

TABLE 3.—*Weight and germination of mature alfalfa seed from noninoculated plants and from plants inoculated with Stemphylium botryosum*

Clones or varieties	Inoculated plants			Check plants		
	Average weight in mg	Percentage germination	Percentage infected seed	Average weight in mg	Percentage germination	Percentage infected seed
Grimm	1.3	52	30	2.9	77	0
Nebr. 2235	1.1	60	26	3.0	75	0
M 18	0.9	44	27	3.1	68	0
L 17	1.2	41	20	3.4	80	0

botryosum were demonstrated also by the reactions of alfalfa clones and varieties to conidial isolates from alfalfa. Some isolates consistently produced only punctate lesions which developed no further, whereas other isolates produced large irregular lesions on the same clones in 8 days. Conidial isolates could not be differentiated from one another on all clones and varieties.

EFFECT OF ENVIRONMENT ON STEMPHYLIUM LEAFSPOT.—Optimum conditions for primary infection were temperatures of 18–22°C. with a relative humidity approaching 100 per cent for at least 12 hours. When the temperature was 22–27°C. for sustained periods, a longer period of high relative humidity was needed for maximum infection. Temperatures above 27°C. reduced the amount of primary infection regardless of relative humidity and there was no infection above 32°C.

Some of the possible explanations for the rather specific temperature and moisture requirements were found in studies of spore germination and penetration. Spores germinated and penetrated leaflets in 8 hours at 22°C., but symptoms appeared only on plants that had been incubated for at least 12 hours at high relative humidity. Spores germinated and penetrated leaflets in 10 to 12 hours at 24–27°C., but longer periods of high relative humidity were required for symptom expression, suggesting a moisture requirement extending for some time after penetration. Temperatures above 32°C. inhibited spore germination.

The effect of temperature during and following incubation was studied. Three sets of 9 plants each were inoculated with conidial suspensions and incubated at 100 per cent relative humidity for 24 hours at 18°, 24°, and 29°C. Following incubation, 3 plants of each set were kept at the same 3 temperatures for 9 days. Plants incubated at 18°C. showed severe symptoms after a 9-day period at 18°C. The other plants incubated at 18°C. showed much less severe symptoms when kept at 24° and 29°C. for 9 days. Plants incubated at 24°C. developed a few scattered large lesions when kept at 18°C. following incubation, scattered red-ring lesions at 24°C. and no infection at 29°C. Plants incubated at 29°C. had few lesions when kept at 18°C. following incubation and none at 24° and 29°C.

That a certain balance between moisture and temperature is necessary for infection to occur was demonstrated further in field tests. When field inoculations were made during warm, dry days and when the days following inoculation were warm and dry, infections developed only on plants in areas where a high relative humidity had been maintained artificially by plastic tents and water sprays. Under the same conditions, plants that were inoculated and left uncovered showed no symptoms. When humid days followed inoculation, and temperatures consistently remained below 27°C., plants beneath the plastic tents were severely diseased, while the lesions that had developed on the plants in the uncovered areas were still in the red-ring stage. Under such conditions, infection was both inhibited and retarded and thus symptom expression was delayed.

Temperature was the most important factor limiting disease development following incubation. Lesions were progressively smaller as the temperature was increased above 22°C. Temperatures above 29°C. inhibited symptom expression, regardless of the conditions during incubation.

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LETTUCE MOSAIC VIRUS TRANSMISSION BY THE GREEN PEACH APHID[1]

Edward S. Sylvester[2]

SUMMARY

In experiments with Great Lakes variety of lettuce on the transmission of lettuce mosaic virus by the green peach aphid, *Myzus persicae* (Sulzer), the results indicated an acquisition threshold period between 11 and 15 seconds. There was a linear gain in efficiency with increasing length of acquisition feeding periods up to 30 seconds. An exponential gain in transmission efficiency occurred with increasing periods of fasting from 5 to 240 minutes prior to an acquisition feeding.

Virus was retained longer by fasting than by feeding vectors. Increasing access time on a virus source from 5 to 120 minutes caused an exponential decrease in vector efficiency, but the rate of infectivity loss was slower than when comparable periods of time were spent on a virus-free host plant.

Serial transmission was possible with brief test feedings, and the number of infections per infective aphid followed either a binominal or Poisson distribution with a mean of 1.3 (range 1 to 4) plants infected per aphid. The area (leaf or cotyledon) or surface (upper or lower) of the test plant fed upon did not affect transmission.

Lowering the temperature decreased the effect of preliminary fasting on subsequent transmission. Allowing an aphid to penetrate healthy tissue prior to an acquisition feeding did not reduce the probability of the aphid becoming infective. Alates were found to be less efficient vectors than either apterae or nymphs.

Virus transmission by groups of aphids followed a binominal expectancy curve. Groups of at least 50 noninfective aphids feeding on lettuce seedlings for 24 hours prior to inoculation of the plants with lettuce mosaic virus by single green peach aphids did not reduce the susceptibility of the test plants significantly. Some evidence was found to indicate that the host plant upon which the aphids are reared may influence virus transmission. Placing test plants in the dark for 48 hours prior to inoculation increased the susceptibility to infection by aphid inoculation. Pretreatment of virus source plants with a short period of cold and/or darkness did not affect the plants as virus sources. Trials with test plants 1–5 weeks of age resulted in more infections in the older plants than in the younger. It was not determined whether the results were due to increased susceptibility with age, or to a greater probability of aphids feeding on large plants. The data as a whole established that the vector-virus relationships between lettuce mosaic virus and the green peach aphid are a nonpersistent type.

Although lettuce, *Lactuca sativa* L., is susceptible to infection by several viruses (18, 21, 27, 29, 33, 59, 60, 67), it is probably the aphid-borne (28) and seed-borne (42) lettuce mosaic virus that causes the most general concern where commercial lettuce crops are grown. Lettuce mosaic virus presumably is world wide in distribution, and in recent years has caused economic losses in lettuce-producing areas of the United States (23) and Great Britain (1, 15). While the host range of lettuce mosaic virus includes a number of weed and ornamental plants (23, 66), seed-borne virus in lettuce is considered to be the important primary source of infection in commercial lettuce fields (1, 15, 20, 23, 26).

The principle aphid vector of the virus in the field is thought to be the green peach aphid, *Myzus persicae* (Sulzer) (15), although other aphids, including *Macrosiphum* (*Dactynotus*) *sonchi* (L.) (36), *M. solanifolii* (Ash.) (1, 15) (= *M. gei* (Koch) *im partim*, = *M. euphorbiae* (Thomas)), *Aphis gossypii* Glover (20), and possibly *Nasonovia ribis-nigri* (Mosley) (11) (= *Macrosiphum hieracii* v. d. G. = *Myzus lactucae* (Schrank)), have been demonstrated experimentally to be vectors. Research on the vector-virus relationships of lettuce mosaic has been minor (20, 33), but it has been established that the basic relationship is nonpersistent (65). Because of the importance of lettuce mosaic in California (23), and the need for more data on nonpersistent vector-virus relationships, the following work was undertaken.

MATERIALS AND METHODS.—The original virus source was plants infected by seed-borne virus. They were grown from commercial seed, variety Great Lakes. Later virus source plants were subinoculations from these plants. Virus-free seed for the test plants was furnished by R. G. Grogan and J. E. Welch, University of California, Davis, California, and was from Great Lakes lettuce seed grown on the Wilbur Ranch in California in 1951. The seeds were germinated in sterilized sand, and seedlings were transplanted into sterilized soil in 3-in. clay pots. Individual aphid transfers were made with a camel's-hair brush, and groups of aphids were collected with an aspirator. When feeding periods were in seconds, the aphids were watched with a hand lens and timed with a stop watch. Whenever an access time was used, a group of

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previously noninfective aphids was placed on a virus source plant and allowed to stay for a 4- or 5-minute period. Previous data (58) have indicated that aphids make about 4 or 5 penetrations, the majority of which are more than 15 seconds in length, during a 5-minute access period. At the end of the test feeding period the aphids were removed or accounted for, and the plants were fumigated with nicotine prior to being placed in the greenhouse for observation. The greenhouses were routinely sprayed at weekly intervals to prevent aphid build-up. Noninfective colonies of aphids were maintained on sugar beet (*Beta vulgaris* L.), White Icicle radish (*Raphanus sativus* L.), or mustard (*Brassica juncea* Coss).

In most experiments, the test plants were in the cotyledon or 1-leaf stage, and during the test feeding period the plants were enclosed under 32 × 9 mm glass vials. Where variable time intervals are listed the figures indicate the mean with its standard deviation. When results are given as ratios, e.g. 5/100, the numerator is the number of plants infected, the denominator is the number of plants tested.

RESULTS.—*Acquisition threshold period.*—Noninfective apterae or late instar nymphs, reared on radish, were allowed to penetrate diseased tissue for the following time intervals: 5, 10, 15, 20, 25, or 30 seconds. A zero time was included to check on possible seedborne virus. Following the acquisition feeding period, single insects were transferred to lettuce seedlings for a test period. All acquisition feeding penetrations were forcibly or mechanically ended, and the 5-, 10-, 15-, and 20-second intervals were randomized in order of use. The 25- and 30-second intervals, the most difficult to achieve, were done alternately first and last during the various replications. Eleven replications were completed, 5 with 8 plants per treatment and 6 with 10 plants per treatment. Two virus source plants were used, 1 for 7 replications and 1 for 4 replications.

The mean of preliminary fasting time was 1.5 ± 0.3 hours and the range 1.0 to 2.2 hours. Analysis of variance indicated that variation among these treatments was not significant. The following are the values for the test feeding periods: 5-second acquisition feeding, 1.5 ± 0.3 hours; 10-second, 1.6 ± 0.3 hours; 15-second, 1.7 ± 0.2 hours; 20-second, 1.4 ± 0.2 hours; 25-second, 1.4 ± 0.2 hours; 30-second, 1.3 ± 0.3 hours. A significant difference among the test feeding periods was indicated ($F = 3.77$, $P = < 0.01$), but it is doubtful if the results on acquisition were influenced appreciably by the small but consistent differences attributable to variation in the test feeding periods used.

The following are the results: 0-second acquisition feeding, 0/100 (number of plants infected/number of plants tested); 5-second, 0/100; 10-second, 0/100; 15-second, 3/99; 20-second, 6/100; 25-second, 9/100; and 30-second, 14/100. Denominators of less than 100 indicate that a test plant died before symptom development.

The acquisition threshold period, under the conditions used, was between 10 and 15 seconds, and approximated the values determined for some other nonpersistent viruses (2, 6, 8, 10, 25, 29, 49, 51, 52, 53). Increase in efficiency with increase in length of acquisition period was linear within the range tested, with no pronounced downward deflection in the 25- or 30-second range. This was in contrast to previous reports by Sylvester (53, 54). However the previous work failed to consider the detrimental influence of artificially ending the acquisition penetration upon virus acquisition (6). If the tested intervals had been extended, it seems probable that a decrease in efficiency would have occurred in the 1- to 2-minute range (6, 10). Most other reports on acquisition threshold periods of nonpersistent viruses indicate that virus can be acquired within 10 seconds. The failure of *M. persicae* to do so in the present work may indicate that the insects required a slightly longer time to penetrate the cuticle and epidermal cell wall when feeding on lettuce leaves than when feeding on the plants used in the earlier work, or that distribution of the virus was such that the low probability of obtaining virus precluded a positive result in the 10-second range with the numbers used. Evidence that the latter is true was obtained in other experiments in the present work in which virus was acquired with an 8-second penetration.

Inoculation threshold period.—Fasted apterae or late instar nymphs were allowed artificially-terminated acquisition feeding on a virus source plant and then were transferred singly to lettuce seedlings for a single test-feeding penetration of either 5, 10, 15, 20, 25, or 30 seconds. A zero-time check also was included. Ten replications, 10 plants per treatment, were done and the order of use of the particular test-feeding interval was randomized. Two virus source plants were used, 1 for 8 replications and 1 for 2 replications. Aphids were reared in the noninfective state on beet, radish, or mustard. The mean of the preliminary fasting time was 2.4 ± 0.6 hours and the range 1.0 to 3.7 hours. The variation among treatments was not significant.

The following are the results: 0-second inoculation feeding, 0/100 (number of plants infected/number of plants tested); 5-second, 0/100; 10-second, 3/99; 15-second, 3/100; 20-second, 1/99; 25-second, 2/100; and 30-second, 2/99.

The efficiency of transmission was low and if conclusions are justified the indications were that no increase in efficiency occurred with increase in the test feeding period up to and including 30 seconds. The low efficiency of transmission was partially due to the controlled artificially-ended 20-second acquisition feeding. The test feedings also were artificially terminated, but with other nonpersistent viruses this treatment has not reduced the probability of infection (6, 58). As with other nonpersistent viruses the minimum inoculation threshold period value was more readily established than was a minimum acquisition threshold period (41, 53, 54). This may indicate that virus

acquisition and virus inoculation are not associated with identical stylet functions. Presumably the acquisition of virus is associated with the intake of cell fluids, with uncertainity as to the role of the salivary sheath in this process, while inoculation presumably is associated with a more or less mechanical act of cell penetration by the stylets (3, 6, 58). Sheath formation probably occurs in both acquisition and inoculation.

Presumably a transmission threshold period value could be established in the 30-second range (25, 52, 53, 54), but the probability of establishing such a value is relatively low, as indicated by the inoculation threshold tests.

Effect of preliminary fasting on transmission.—In transmission of a typical nonpersistent virus, vector efficiency can be increased by fasting aphids prior to acquisition feeding (17, 25, 32, 37, 38, 40, 41, 53, 54, 58, 62, 63, 65). In general, efficiency gained is correlated positively with length of the preliminary fasting period. When transmission efficiency is high, benefits of preliminary fasting are readily demonstrable even with brief fasting periods, e.g., within 15 minutes, and there is little gain in efficiency thereafter (17, 25, 53). However, when efficiency of transmission is low, increase in efficiency may proceed at a slower rate, and usually it is a matter of hours before the maximum effects of preliminary fasting are obtained (55, 58, 62, 63).

Data on the effects of preliminary fasting on the efficiency of transmission of lettuce mosaic virus by *M. persicae* were obtained in the following experiment which was replicated 10 times, with 10 plants per treatment. Three different virus source plants were used—1 for 5 replications, 1 for 3 replications, and 1 for 2 replications. Lots of 20 feeding green peach aphid apterae or late instar nymphs, reared on beet, were aspirated into glass vials. Following a fasting period in the vial of 0, 5, 10, 15, 30, 60, 120, 180, or 240 minutes, the insects were placed on a virus source leaf and allowed to remain for a period of 5 minutes. At the end of the access time, the aphids were aspirated into a vial and 10 aphids were selected at random from the group and placed singly on healthy lettuce seedings. One to 2 minutes were required to transfer the 10 insects from the source plant to the test plants. The mean of the test feeding periods was 1.02 ± 0.06 hours and the range 1.0 to 1.1 hours. The effect of this variation among treatments on results was not significant.

The following are the results: 0-minute preliminary fastings, 0/99 (number of plants infected/number of plants tested); 5-minute, 6/96; 10-minute, 8/97; 15-minute, 7/99; 30-minute, 15/99; 60-minute, 19/100; 120-minute, 22/100; 180-minute, 18/99; and 240-minute, 31/97.

The type of curve which could be derived is exponential with a response to preliminary fasting detectable within 5 minutes. The most rapid gain in efficiency occurred within the first 30 minutes of fasting, with subsequent increase evident but at a decreased rate up to 4 hours. The relatively low value obtained at the 3-hour interval was probably due to sample variation, and it would be difficult to attach any particular significance to the deflection. Whether or not the maximum influence of fasting was demonstrated by the test was not determined, but the indications were that further increase in efficiency would have been slight if the preliminary fasting time was extended beyond 4 hours.

At the end of the test feeding period, a certain proportion of the insects were found not to be feeding on the test plants. It is difficult to judge whether or not these insects fed on the test plants, for only when a positive result occurred was there conclusive evidence of feeding. One method to circumvent the difficulty is to utilize only those data from plants which had aphids on them at the end of the test feeding. When this is done the results are as follows: 0 minutes preliminary fasting, 0 per cent (0/48); 5 minutes, 10.6 per cent (5/47); 10 minutes, 16.2 per cent (7/43); 15 minutes, 13.1 per cent (6/46); 30 minutes, 20 per cent (10/50); 60 minutes, 27.5 per cent (14/51); 120 minutes, 31 per cent (17/55); 180 minutes, 28.3 per cent (15/53); 240 minutes, 45 per cent (23/51). When the data are expressed graphically, the curves from the corrected and uncorrected data parallel one another, indicating that the effect of the variable introduced by the tendency of aphids to wander off test plants was linear throughout the treatments.

Effect of postfasting on virus retention.—Regardless of whether an infective aphid is feeding or fasting after acquisition of a nonpersistent virus, there is a loss of infectivity. If infective insects are fasted after virus acquisition, the probability of obtaining an infection decreases as the length of postacquisition fasting increases. Data establishing this phenomenon have been obtained with several nonpersistent viruses (5, 8, 18, 22, 25, 27, 31, 33, 37, 38, 39, 41, 42, 53, 58, 62, 63, 64). Some data have been obtained that indicate an effect of temperature upon the rate of loss of efficiency (8, 31, 58).

The following experiment of 15 replications, 10 plants per treatment, was done to determine the rate of loss of infectivity under conditions of postacquisition fasting at room temperature, approximately 22°C. Four virus source plants were used—1 for 9 replications, 1 for 1 replication, and 2 each for 2 replications. Groups of green peach aphid apterae or late instar nymphs (reared on healthy sugar beet, radish, or mustard plants) were given a preliminary fasting, were allowed a 4- to 5-minute access time on a virus source leaf, and then were aspirated into vials and fasted for 0, 5, 10, 15, 30, 60, 120, 180, or 240 minutes. Single aphids then were transferred to healthy lettuce seedlings for a test feeding period.

The mean value for the preliminary fasting times was 1.1 ± 0.07 hours and the range 1.0 to 1.3 hours.

The mean value for the test feeding time was 1.1 ± 0.09 hours and the range 1.0 to 1.7 hours. In both cases the variation among treatments was not significant.

The following are the results: 0 minutes postacquisition fasting, 24/150 (number of plants infected/number of plants tested); 5 minutes, 25/150; 10 minutes, 16/150; 15 minutes, 12/150; 30 minutes, 13/150; 60 minutes, 11/150; 120 minutes, 4/150; 180 minutes, 1/150; and 240 minutes, 4/150.

The aphids began to lose virus charge soon after acquisition. The greatest loss occurred within the first 30 minutes, and the rate was somewhat exponential. It is not known whether virus in a fasting vector is inactivated by *in vitro* exposure to substances of insect origin, or is mechanically dispersed by either ejection or ingestion. If under the conditions of the experiments the virus is considered to undergo a constant rate of diminution (51) such that half the activity is reduced each 30 minutes, the expected and actual results if plotted graphically would follow the same general curve with the inevitable variations present in biological data gathered through small samples.

As in the previous experiment, some of the aphids were not on the test plants at the end of the test feeding period. There was a tendency for the aphids fasted for the longer intervals to be less restless. When the data were corrected by using only results from plants which had aphids on them at the end of the test feeding period, the following transmission results were obtained: 0 minutes postacquisition fasting, 21.9 per cent (16/73); 5 minutes, 19.6 per cent (20/102); 10 minutes, 13.3 per cent (13/98); 15 minutes, 9.2 per cent (9/98); 30 minutes, 11.6 per cent (11/95); 60 minutes, 8.5 per cent (10/117); 120 minutes, 3.5 per cent (4/113); 180 minutes, 0.85 per cent (1/117); and 240 minutes, 3.2 per cent (4/126). Expressed graphically, the corrected and uncorrected results follow the same general curve, the difference again being one of degree.

Loss of infectivity by feeding insects.—Nonpersistent viruses are characteristically lost by infective insects within relatively short periods of time. The previous experiment indicated the rate of loss of infectivity by fasting infective aphids. Infectivity also is lost by feeding insects, and this occurs whether the insects are feeding on a virus source plant or on a healthy plant. The rate of loss is greater when insects are feeding than when they are fasting (6, 8, 17, 25, 31, 37, 38, 39, 40, 58, 61, 62, 63). In the present work, data were obtained upon the existence of these phenomena in the case of the green peach aphid and lettuce mosaic virus. In one experiment, the influence of prolonging the access periods on vector efficiency was determined, and in another, data were obtained on the effect of prolonging the test feeding period on subsequent transmission to a second healthy plant.

Different lots of fasted, noninfective apterae or late instar nymphs, reared on beet, radish, or mustard, were allowed access to a virus source for 1 naturally-terminated penetration (mean value = 24.9 ± 9.5 seconds), or for 5, 10, 15, 30, 60, or 120 minutes. The insects then were caged singly on lettuce seedlings for a test feeding. The means of preliminary fasting periods and of the test feeding periods were 1.3 ± 0.2 and 1.1 ± 0.2 hours, respectively, and the ranges 0.9 to 1.8 and 0.8 to 2.1 hours, respectively. In neither case was variation among treatments significant. The experiment was replicated 10 times with 10 plants per treatment and with 4 different virus sources (1 for 5 replications, 1 for 4 replications, 1 for 3 replications, and 1 for 1 replication).

The following are the results: 1 normal penetration, 18/100 (number of plants infected/number of plants tested); 5 minutes access time, 14/100; 10 minutes, 10/100; 15 minutes, 11/100; 30 minutes, 2/99; 60 minutes, 4/100; and 120 minutes, 3/99.

The initial transmission efficiency (1 normally-ended feeding) of 18 per cent was reduced to the 2–4 per cent range within 30 minutes. The rate of infectivity loss was faster than in fasting insects. The results could be interpreted as indicating that virus was being inactivated (64), that the rate of feeding was progressively reduced (65), or that less virus was available in the deeper tissues of the plants (3, 6, 7, 8, 9, 10). Recent evidence (3, 9) indicates that perhaps the last named is quite probable since the region from which insects can pick up certain viruses most efficiently appears to be the epidermis.

Data rectified for missing aphids parallel the uncorrected data reasonably well, the transmission results being: 1 normal penetration, 31.6 per cent (12/38); 5 minutes access time, 19.4 per cent (7/36); 10 minutes, 18.9 per cent (7/37); 15 minutes, 20 per cent (8/40); 30 minutes, 4.35 per cent (2/46); 60 minutes, 8.8 per cent (3/34); and 120 minutes, 2.76 per cent (1/36).

If the amount of transmission is correlated with the rate of penetration (minimum of 15 minutes to reach the phloem (46)) and if the tissue penetrated varies with respect to the amount of virus available to the insects, the technique of varying the access time might be used to sample and assay various tissue regions of the plant to determine the available amounts of insect-transmissible virus. If, however, along with other factors, there is an additional complication due to virus inactivation by insect contact (63, 65), it will be increasingly difficult to interpret the results on the basis of available virus in various tissue regions.

Another method of testing the influence of feeding on loss of efficiency by infective insects is to allow them to feed on a healthy plant for a varying length of time before they are tested for infectivity on a final test plant (8, 17, 31, 58, 61, 65). The rate of loss of lettuce mosaic virus in feeding apterae or late instar nymphs of *M. persicae* was determined in an experiment in which 20 replications and 10 plants per treatment were used. Two virus source plants were

used, 1 for 14 replications and 1 for 6 replications. Lots of 150 noninfective aphids, reared on either radish or mustard plants, were fasted in a vial and then placed on a virus source leaf for an access period of 4–5 minutes. At the end of the access time, the insects were aspirated off and placed on a healthy lettuce leaf for periods of 0, 5, 10, 15, 30, 60, 120, 180, or 240 minutes. At the end of the feeding time on the first healthy leaf, the aphids were removed by aspiration in lots of 10 and placed singly on healthy lettuce seedlings for a test feeding period.

The mean for the preliminary fasting periods was 1.3 ± 0.3 hours and the range 1.0 to 2.5 hours. Variation among treatments was not significant. The mean for the test feeding periods was 1.02 ± 0.02 hours and the range 0.9 to 1.1 hours. Analysis of variance indicated a significant variation among treatments, but it is doubtful that variation of 2 to 3 minutes in test feeding time significantly affected the results.

The following are the results: 0 minutes access time on a healthy plant before the test feeding, 26/200 (number of plants infected/number of plants tested); 5 minutes access time, 11/200; 10 minutes, 2/200; 15 minutes, 2/200; 30 minutes, 3/200; and 60, 120, 180, or 240 minutes, 0/200.

In these tests, loss of infectivity occurred at a much faster rate than when insects were allowed comparable access time on a virus source plant. This probably reflected the rate of dispersion of virus into healthy tissue and is associated with feeding activities. Dispersion could be by ejection with the saliva and/or ingestion with the normal food material. Again the rate appeared to be exponential. When the data were adjusted to include only those plants upon which aphids were present at the end of the test feeding period, the results were as follows: 0 minutes access time on a healthy plant, 21.5 per cent (21/99); 5 minutes, 12.5 per cent (11/88); 10 minutes, 2.24 per cent (2/89); 15 minutes, 2.44 per cent (2/82); 30 minutes, 3.22 per cent (3/93); and 60, 120, 180, or 240 minutes, 0 per cent.

In summary, it appears that the rate of loss is more rapid under conditions of feeding than under conditions of fasting, and that infective aphids lose infectivity more rapidly when feeding on healthy plants than when feeding on a virus source plant. Regardless of treatment, it is apparent that the greatest potential for vector infectivity occurs within a few minutes after fasted insects have acquired a virus charge by a brief penetration of diseased tissue.

Serial transmission.—It is well established that aphid vectors of nonpersistent viruses can cause infections in more than 1 plant after leaving a virus source (6, 8, 10, 37, 38, 39, 58, 61, 62, 63, 65). The number of plants that become infected appears to vary with the particular vector-virus combination. To evaluate the capacity of green peach aphid apterae to transmit serially lettuce mosaic virus, 139 individuals were tested under the following conditions. Single aphids, reared variously on sugar beets, mustard, or radish plants, were placed in a vial for a preliminary fasting period of 3.88 ± 1.94 hours. After the fast they were placed individually on a virus source plant and allowed to make single normally-ended penetrations averaging 23.84 ± 10.36 seconds. The restriction placed on the length of the penetration was that it be normally terminated by the aphid within 60 seconds. Aphids which fed over 60 seconds were discarded. Seventeen aphids out of 156 used were discarded for this reason. Following the acquisition feeding each insect was transferred to a healthy lettuce seedling and allowed to make a single 15-second penetration. It was then moved to a second healthy plant and again allowed one 15-second penetration. The procedure was repeated until the aphid was stimulated to a point where it spent an excessive amount of time without attempting to penetrate the test plant, or was too weak to cooperate, or had fed on a series of 20 plants. If the test feeding was normally interrupted in less than 15 seconds the insect was moved to the next plant. The mean value for the test feedings was 14.6 ± 1.33 seconds, based upon a total of 1804 recorded penetrations. It required an average of 1.39 ± 0.54 minutes for each aphid to be transferred, to become oriented, and to feed on a test plant.

The actual tests were made in 12 series, each on a different day. Two virus source plants were used each for 5 series, and a third virus source plant was used for the final 2 series. A record was kept of the site of feeding.

The results of the trials are given in Tables 1 and 2. Thirty-seven out of 139 insects proved to be infective (26.62 per cent), and these insects infected a total of 48 out of a possible 1807 test plants (2.65 per cent transmission). The mean number of plants infected per aphid was 1.30, with a range of 1 to 4. The average number of plants fed upon by each aphid was approximately 12, and if the infective insects were approximately equal in virus charge, the chances of an insect infecting any given number of plants up to and including 12 should follow a binominal distribution $(p + q)^{12}$ or a Poisson distribution. The results of such calculations (Table 2) indicate that the aphids were somewhat uniformly infective (7). The probabilities of the first, second, third, or fourth plant fed upon becoming infected were about equal, after which there was a decline in the likelihood of infection (Table 1). The longest time that an insect remained infective was when the thirteenth plant fed upon by 1 aphid became infected, and in this case no plant previously fed upon became infected.

As in previous work (56) the area of the plant that was inoculated did not appear to affect the results significantly (Table 3). In general the area of the plant most likely to be fed upon was correlated with incidence of infection. One exception to this (excluding the "junction," but in this case the sample was very small) was in the case of the upper cotyledon

TABLE 1.—*Distribution of infections by lettuce mosaic virus when single infective apterae were fasted 2–6 hours, allowed 1 normally-ended acquisition penetration, and then transferred singly to a series of healthy lettuce seedlings and allowed one 15-second test feeding penetration on each*

Successive plant number fed upon[a]																				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Frequency[b]
+	−	−	×																	1
+	−	−	−	−	−	−	×													2
+	−	−	−	−	−	−	−	−	−	−	−	×								1
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×				1
+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
+	+	+	+	−	−	−	−	−	−	−	−	×								1
+	−	+	−	−	−	−	−	−	−	×										1
+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×		1
−	+	−	−	−	−	−	×													1
−	+	−	−	−	−	−	−	×												1
−	+	−	−	−	−	−	−	−	−	−	−	×								1
−	+	−	−	−	−	−	−	−	−	−	−	−	×							1
−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	3
−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	×					1
−	−	+	−	−	−	−	−	×												1
−	−	+	−	−	−	−	−	−	−	×										2
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	×					1
−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	+	+	−	+	−	−	×												1
−	−	−	+	−	−	−	−	−	−	−	×									1
−	−	−	+	−	−	−	−	−	−	−	−	×								2
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	1
−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	+	−	−	−	−	×											1
−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	2
−	−	−	−	−	−	+	−	−	−	−	−	−	×							1
−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	×			1
−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	1
−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	1

[a] Positive sign (+) indicates infection; negative sign (−) means no infection. The × sign indicates discontinuance of transfers because of poor condition of aphid.
[b] Frequency refers to number of times particular distribution occurred.

surface which was fed upon by 9.31 per cent of the insects and yet 18.75 per cent of the transmissions occurred when the insects fed at this site. The most probable explanation of this is that a bias was introduced. The insects were placed initially on the upper surface of the cotyledon or leaf, depending upon the stage of seedling development, and since the insects had just finished a fasting period and had but 1 opportunity to feed before being tested, they tended to feed where placed. Later, as the aphids had been moved several times they became more restless and moved further before attempting to feed. Since the greatest probability for infection occurred among the first 4 plants fed upon, a bias was introduced. A summary of the data for the first 4 plants only shows that the upper surface, including that of both leaf and cotyledon, was the site fed upon by 48 per cent of the insects and these feedings resulted in 44.4 per cent of the total number of infections. The lower surface was fed upon in 42 per cent of the cases, resulting in 41.6 per cent of the transmissions. Thus the factor of probability of infection within the series, as well as the probability of a particular site being fed upon within the series, must be considered when attempting to draw inferences suggested by the data.

Effect of temperature on increased efficiency resulting from preliminary fasting.—Although such data with nonpersistent viruses are not extensive, the general trend has been that lowering the temperature adversely affects the gain in transmission efficiency due

TABLE 2.—*Summarization[a] of data on serial transmission of lettuce mosaic virus by single apterae that had been fasted for 2–6 hours, allowed 1 normally-ended acquisition penetration, and then allowed one 15-second test feeding penetration of each lettuce plant fed upon*

Infections per aphid	Distribution of infections: Expected		Actual
	Binominal	Poisson	
0	100.08	98.44	102
1	33.86	34.6	29
2	5.14	5.85	6
3	0.47	0.69	1
4 or more	0.027	0.08	1

[a] A total of 37 out of 139 aphids tested were infective and these caused infections in 48 out of 1807 plants fed upon, with a mean of 1.3 ± 0.67 plants infected per aphid.

TABLE 3.—*Data gathered in connection with serial transmission experiments summarized according to the site of inoculation of healthy lettuce seedlings with lettuce mosaic virus by apterae of M. persicae*

	Site of inoculation[a]								
	UC	UL	LC	LL	S	J	LP	CP	YL
	%	%	%	%	%	%	%	%	%
Feedings[b]	9.31	21.60	18.63	35.04	0.28	0.45	2.95	4.83	0.91
Transmissions[c]	18.75	22.9	10.4	37.6	0.00	4.16	2.08	4.15	0.00

[a] The symbols represent the following areas: UC—upper cotyledon; UL—upper leaf; LC—lower cotyledon; LL—lower leaf; S—stem; J—junction of stem, cotyledon and leaf petioles; LP—leaf petiole; CP—cotyledon petiole; YL—youngest, not yet unfolded.
[b] Percentage of plants that were fed upon at indicated sites. Figures are based on records from 1760 plants.
[c] Percentage of total number of transmissions (48) that resulted from feedings at indicated sites.

to preacquisition fasting (58). To determine the effects of lowering the temperature with lettuce mosaic virus and the green peach aphid, the following experiment was replicated 5 times with 20 plants per treatment. Two virus source plants were used, 1 for 3 replications and another for 2 replications. A group of aphids, aspirated from a noninfected mustard plant into a vial, were allowed a preliminary fasting period at room temperature of 0, 30, 60, 120, 180, or 240 minutes. A similar group was fasted in a refrigerator for similar intervals of time (excluding the 0 time interval). At the end of the preacquisition fasting period the group was placed on a virus source leaf for 4–5 minutes, and then collected by aspiration and placed singly on healthy lettuce seedlings for a test feeding. The laboratory temperature during the preacquisition fasting period was 23.14 ± 0.84°C., while that of the refrigerator was 6.09 ± 0.92°C. The mean for the test feeding periods was 1.04 ± 0.07 hours and the range 0.9 to 1.2 hours. The variation among treatments was not significant.

About 8 per cent transmission was obtained with nonfasted insects (Table 4). After a preacquisition fasting period of 30 minutes, either at room or refrigerator temperature, transmission was approximately 25 per cent. With aphids fasted at room temperature, efficiency continued to increase as the fasting period was lengthened with a maximum transmission efficiency occurring in the 2- to 4-hour range. In contrast, aphids fasted before acquisition at the lower temperature failed to show any increase in efficiency with increasing fasting time; rather there was a decrease in efficiency at all intervals when compared to the 30-minute intervals. Similar results were obtained with the Brassica nigra virus (58). The reason for this decline is not known, but perhaps the low temperature made the insects sluggish and the 4- to 5-minute access time at room temperature was insufficient for the insects to regain normal feeding activity. If this is true, the data would indicate that 30 minutes at approximately 6°C. is not long enough to affect the normal feeding activity of the insects. Correction for aphids missing from the test plants failed to change the trend of the results.

In another experiment, insects were allowed time for possible recovery after a preacquisition fasting period in the refrigerator. Four replications were used with 25 plants per treatment and 2 virus source plants, each for 2 replications. Single aphids, reared on sugar beets, were fasted for either 30 minutes or 4 hours at room temperature (23.31 ± 0.48°C) and compared with aphids fasted for 3.5 hours in a refrigerator (5.78 ± 0.79°C) and then returned to room temperature for 30 minutes prior to a 4- to 5-minute access time on a virus source plant. The mean of the test feeding periods was 1.0 ± 0.02 hours and the range was 0.98 to 1.2 hours. The variation among treatments was not significant.

In these trials, the ratios of number of plants infected to the number of plants used were as follows: 30 minutes of preliminary fasting at room temperature, 17/100; 4 hours fasting at room temperature, 32/100; and 3.5 hours fasting at approximately 6°C plus 0.5 hours at room temperature, 21/100. The 3.5 hours of preacquisition fasting at low temperature did not greatly increase the vector efficiency. When the data were corrected for aphids missing from the test plants at the end of the test feeding, the results in the same order were: 30-minute fasting at room temperature, 25 per cent (15/59) transmission; 4 hours fasting (room temperature), 37 per cent (27/73) transmission; 3.5 hours fasting (refrigerator) plus 0.5 hours (room), 26 per cent (17/65) transmission. Ap-

TABLE 4.—*Transmission of lettuce mosaic virus by single aphids allowed variable preliminary fasting times at 2 temperatures followed by a 4- to 5-minute access period on a virus source plant, and then allowed a test feeding period of approximately 1 hour on healthy lettuce seedlings*

Minutes of preliminary fasting	Temperature during fasting	
	Room (23 ± 0.8°C)[a]	Refrigerator (6 ± 0.9°C)[a]
0	8/100	———
30	24/100	26/100
60	38/100	13/100
120	48/100	10/100
180	57/100	15/100
240	46/100	10/100

[a] In the figures in these columns, numerator is number of plants infected; denominator, number tested.

parently the fasting of aphids at low temperatures prior to an acquisition feeding did not materially affect the resulting vector efficiency.

Effect of penetration of healthy plant tissue on virus acquisition.—Under field conditions there may be a very low probability that an aphid, after a preliminary fasting period due to flight, will feed on a diseased plant before feeding on a healthy one, especially when the number of diseased plants in the field is small. Consequently it is of interest to determine whether or not the efficiency of transmission is reduced if a recently fasted insect is allowed to penetrate healthy tissue prior to being allowed access to a virus source. Experiments which have been reported on this phenomenon have indicated that there is no material reduction in efficiency (6, 8, 54, 58). Additional data were gathered in the following experiment of 6 replications with 20 plants per treatment and using 1 virus source plant. Noninfective aphids were taken from sugar beet and placed in vials for a preliminary fasting period of 1.6 ± 0.4 (1.0 to 2.4) hours. The variation among treatments was not significant. At the end of the fasting, an individual was selected and allowed to make 1 normally-ended penetration into a healthy lettuce leaf. After the penetration, the insect was transferred to a virus source leaf and allowed to make 1 normally-terminated acquisition penetration after which it was transferred to a healthy lettuce seedling for a test feeding. In the comparison series the fasted individuals were transferred from the fasting vial directly to a virus source leaf and allowed to make 1 normally-interrupted penetration and then were transferred singly to the test plants for a feeding period of 1.2 ± 0.6 (0.7 to 2.9) hours. The duration of the penetration of healthy leaves was 19.13 ± 6.9 seconds, that of the subsequent acquisition feeding was 16.12 ± 6.7 seconds, and that of the check series acquisition feeding was 19.59 ± 7.4 seconds. There was a tendency for the second penetrations to be shorter than the first penetrations.

The aphids that had a single penetration into healthy tissue prior to the acquisition feeding transmitted the virus to 43 out of 120 plants, while those that were allowed immediate access to a virus source following the preliminary fasting transmitted the virus to 40 out of 120 plants. When the data were corrected for aphids which were missing from the test plants at the end of the test feeding period, the results were 38/92 and 39/94 infections, respectively. Allowing fasted aphids a penetration into healthy tissue prior to an acquisition feeding did not reduce the probability of subsequent virus acquisition.

Graphs derived from these data indicate that there is a close correlation between the distribution of the length of penetration and that of the infections resulting. An area of some pronounced deviation is that in the 6- to 10-second range. There was a slightly greater probability of an infection occurring with a 6- to 10-second acquisition pentration than with longer or shorter ones. This occurred in both classes of treatments.

Transmissions by alates, apterae, and nymphs.—In the majority of transmission trials with nonpersistent viruses, it has been found that the particular form of an aphid species used does not greatly influence the transmission efficiency (25, 27, 48). However, the tests have been limited in number. Since apterous individuals are more suitable for transmission work than are other forms, concentration of effort has been with this form.

In the following experiment, replicated 4 times with 25 plants per treatment and with a single virus source plant, the relative efficiencies of alate, apterous, and nymphal forms of *M. persicae* reared as noninfectives on radish were determined. The nymphs used were primarily in the first or second instar. A group of approximately 50 of the particular form of the aphid was aspirated into a vial for a preliminary fasting period of 1.81 ± 0.21 hours (alates), 2.45 ± 0.31 hours (apterae), or 2.36 ± 0.14 hours (nymphs). Following the fasting the insects were placed on a virus source leaf for 4–5 minutes, after which they were collected by aspiration and transferred singly to healthy lettuce seedlings for a test feeding period of 1.57 ± 0.12 hours for the alates, 1.28 ± 0.72 hours for the apterae, and 1.47 ± 0.78 hours for the nymphs.

The ratios of number of plants infected to the number of plants used were 6/100 for alates, 21/99 for apterae, and 21/100 for nymphs. The apterous and nymphal forms transmitted with approximately equal efficiency, but the alates were approximately ⅓ as efficient. A similar trend was obtained by Hamlyn (25).

Of immediate concern in such an experimental design is the problem of alate activity, consequently the results were evaluated using only data from aphids that were actually present on the test plants at the end of the test feeding period. In this case the ratios were 6/49 with alates, 15/42 with apterae and 15/46 with nymphs. Even with the additional restriction, the alate transmission efficiency was approximately 1/3 that of either the apterae or nymphs. Again, the apterae and nymphs transmitted with approximately equal efficiency. It appears that the problem of comparative transmission by forms of a given aphid species needs some additional assessment. This is especially true since most of the laboratory results are concerned with apterous transmission while it is generally held that alates are primarily concerned with the field spread of nonpersistent plant viruses (13, 14, 24, 34, 64).

Influence of number of aphids on transmission.—Throughout the literature are numerous reports on the transmission efficiency of vector groups (27, 37, 38, 61, 63, 65). The general conclusion has been that transmission efficiency by groups of insects follows that predicted by probabilities of a binominal distribution (61). The recent exception to this is the work of Kirkpatrick and Ross (35) in which evidence was

presented to indicate that the actual transmission of potato leafroll virus by groups of aphids was less than that predicted by the binominal distribution. The interpretation was that insect feeding reduced plant susceptibility to infection. This work has not yet been confirmed and was concerned with a persistent aphid-borne virus and involved use of long test feedings.

The following experiments evaluated the transmission of lettuce mosaic virus by groups of 1, 2, 3, 4, or 5 green peach aphid apterae or late instar nymphs. The insects were from 1 colony reared on sugar beet. One virus source plant was used. Aphids were collected in vials in lots of 100 by aspiration and given a preliminary fasting period of 2.19 ± 0.24 hours. Following the fasting, the insects were placed in groups of 100 on a virus source leaf for 4–5 minutes after which they were collected in a vial by aspiration and then transferred by brush to test plants in groups of 1, 2, 3, 4, or 5 insects per plant. The procedure was repeated until 20 plants had been used for each treatment, and the entire cycle was done on 5 different occasions. If a single aphid was placed on the first plant then the next plant had 2 aphids placed on it, the next 3, the next 4, and the last 5. This procedure was continued until the supply of infective aphids in a particular vial was exhausted, then another vial was taken. In the first replication, the first plant had 1 aphid placed on it, then the 2, 3, 4, or 5 aphids were placed on plants in that order. The order of use of the groups in the next replication was 2, 3, 4, 5, and 1; that of the third replications was 3, 4, 5, 1, and 2; that of the fourth was 4, 5, 1, 2, and 3; and that of the last replication was 5, 1, 2, 3, and 4. This was an attempt to distribute equally the influence of the variable fasting time throughout the treatments and replications. To inoculate any group of 5 plants with 1, 2, 3, 4, and 5 aphids required an average of 2.5 minutes. The test feeding time was 1.12 ± 0.26 hours.

The results of the trials are in Table 5. Use of the relationship of P (probability of obtaining an infection) = $1 - q^x$ (q = probability of not obtaining an infection, and x = number of insects used), shows that the actual transmission and the expected transmission coincide reasonably well. An even closer fit between the actual and the expected is possible if the expected value for 1 aphid is calculated from the data taken from the total sample, and if the results are based upon the mean number of aphids present on the plants at the end of the test feeding periods. Thus no evidence was obtained in support of the hypothesis that increasing the number of insects reduces the susceptibility of the test plants, at least in connection with the transmission of lettuce mosaic virus by the green peach aphid under the laboratory conditions.

Effect of the feeding of noninfective aphids on plant susceptibility.—The hypothesis that aphids (infective or noninfective) feeding on a plant reduce the susceptibility of that plant to infection with a virus was further tested in an experiment in which 80 plants per treatment and 1 virus source plant were used.

TABLE 5.—*Transmission of lettuce mosaic virus by groups of apterae or late instar nymphs given a preliminary fasting of approximately 2 hours, a 4- to 5-minute access time on a virus source, and a test feeding period of approximately 1 hour*

	Percentage infection									
	Uncorrected[a]					Corrected[b]				
	Number of aphids					Number of aphids				
	1	2	3	4	5	0.6	1.4	1.8	2.6	3.3
Actual	26	47	56	55	76	26	47	56	55	76
Expected[c]	26	45	59	70	78	22.1	44.1	52.6	66.0	74.6

[a] Expected results in these columns were calculated on the assumption that 26 per cent of the aphids were capable of causing infections when feeding alone.

[b] Corrected results were calculated from the mean expectancy for infection with 1 aphid based on the results from all groups and from the mean values for the number of aphids actually found on the test plants at the end of the test feedings. Total number of infections, 260 out of 500 plants tested.

[c] Expected results were calculated using $p = 1 - q$ for the probability of obtaining an infection with 1 aphid and $P = 1 - q^x$ for the probability of obtaining infection using x aphids.

A series of 80 seedling lettuce plants in the 1- or 2-leaf stage were each caged and infested with a minimum of 50 noninfective green peach aphids. At the same time a comparable series of 80 plants were placed under the same conditions (caged in the laboratory) but not infested. The noninfective aphids came from 2 colonies, 1 from radish and 1 from beet.

In the afternoon of the same day a series of 200 or more mature apterae were aspirated from a beet colony and each marked on the abdominal dorsum with a spot of India ink. The marked aphids were placed on a young mustard seedling and allowed to feed overnight. The following day the marked aphids were aspirated in lots of 50 and given a preliminary fasting period of 1.80 ± 0.26 hours and then placed on a virus source leaf for a 4- to 5-minute access period. The insects then were again aspirated into a vial and placed singly on each of 160 test plants. The fasting, transfer, and feeding times were staggered so as to inoculate the plants in groups of 20, allowing 4 replications of 20 plants per treatment. After a test feeding of 1.07 ± 0.76 hours the plants were examined and the marked apterae accounted for.

Eleven of the 80 infested plants and 11 of the noninfested ones became infected. When the data were corrected for missing aphids, the results showed that 10 of 68 infested plants and 14 of 66 noninfested plants became infected. (adjusted $\chi^2 = 0.58$, $P = >0.30$). Thus infesting seedling lettuce plants with small populations (approximately 50 insects) of green peach aphids for 24 hours prior to inoculation with single aphids of the same species failed to reduce significantly the susceptibility of the plants to infection by lettuce mosaic virus.

Effect of aphid-rearing host plant on transmission efficiency.—Frequently, it is desirable and necessary

to maintain vector species on a variety of host plants. For example, during the winter months *M. persicae* can be more easily maintained on mustard or radish plants than on sugar beets. It is not known whether or not the kind of plant on which aphids are reared affects subsequent transmission of specific viruses. In the present work the aphids were not reared on a host plant of the virus used, and at various times aphids were selected from colonies on sugar beet, radish, or mustard plants. The following tests were designed to determine if possible the influence of the host plant upon which the vectors were reared on subsequent transmission of lettuce mosaic virus.

Green peach aphids were reared on 3 herbaceous plants: sugar beet, mustard, and radish (variety White Icicle). In addition, green peach aphids from a naturally occurring infestation of *M. persicae* on Lovell peach tree seedlings were used. It is highly probable that the aphids which were reared on the herbaceous hosts were genetically similar, since the stock colony from which the colonies were derived was established a number of years ago at the experiment station. Additional introductions of the species from the field have been relatively few over the years, and consequently it probably must be considered that the stock colonies are a somewhat selected laboratory strain of the species. However, the colony which developed on peach apparently was of different origin and consequently the results of the trials should be intrepretated with the possibility that 2 aphid strains were involved.

The following experiment consisted of a series of 4 replications, 50 plants per treatment. Small populations of aphids were aspirated from a colony of aphids on mustard, sugar beet, radish, or peach, and given a preliminary fasting. The insects then were allowed a 4- to 5-minute access period on a virus source plant, at the end of which they were collected again into a vial by aspiration and then transferred singly by means of a camel's-hair brush to healthy lettuce seedlings. The experimental design was that of a Latin square of 4 replications, 4 virus source plants, and 4 sources of insects. The mean of the preliminary fasting periods was 2.8 ± 0.2 hours and the range 2.5 to 3.3 hours. Those for the test feeding periods were 1.2 ± 0.2 and 1.0 to 1.7 hours, respectively. Variation among treatments in the preliminary fasting and feeding periods were not significant.

Aphids from mustard, beet, and radish transmitted lettuce mosaic virus with similar efficiencies, but those from peach were less efficient (Table 6). There also were differences among virus source plants which accounted for almost as much variation in the experiment as did those attributable to the host plant of the aphids.

Since the possibility of different strains of aphid species existed, it is not certain that the differences found among the colonies were due to the host plants used for rearing the aphids. However, the trend was strong enough to warrant further consideration in the future. Another factor which could have influenced the results was the adaptability of the insects to lettuce as a host plant for the period of time used. The results could be explained partially if the aphids from peach failed to feed readily on lettuce. Records of the aphids present on the test plants at the end of the test feeding period showed that there were more aphids present on the test plants when the aphids came from peach, than from any other host. Thus it would appear that restlessness was not the explanation of the results.

When the results were tabulated using only those plants upon which aphids were present at the end of the test feeding period, the transmissions were as follows: aphids from mustard, 40 per cent (38/95); aphids from beet, 30.6 per cent (30/98); aphids from radish, 30.8 per cent (29/94); aphids from peach, 17.3 per cent (21/121); virus from source A, 24.6 per cent (26/110); virus from source B, 33.4 per cent (29/87); virus from source C, 37.2 per cent (39/105); virus from source D, 22.6 per cent (24/106); replication I, 29 per cent (39/124); replication II, 27.3 per cent (27/99); replication III, 39.2 per cent (42/107); and replication IV, 14.6 per cent (10/68). Summarization of the data in this manner leads to the same conclusion as before, except that the variation due to replication becomes a greater factor.

TABLE 6.—*Transmission of lettuce mosaic virus by aphids that had been reared on 4 different host plants, fasted for 2–3 hours, given a 4- to 5-minute access time on a virus source plant, and then given approximately 1-hour test feedings on lettuce seedlings*

Replication	Host plant used to rear aphids[a]			
	Mustard	Sugar beet	Radish	Peach seedling
I	22/50	13/50	9/50	7/50
II	10/50	8/50	15/50	8/50
III	14/50	18/50	15/50	11/49
IV	12/50	8/50	15/50	2/49
Totals:				
Treatment	58/200	47/200	54/200	28/198
Replication	(I) 51/200	(II) 41/200	(III) 58/199	(IV) 37/199
Virus Source	(A) 40/200	(B) 51/199	(C) 63/200	(D) 33/199
Aphids present[b]	95/200	98/200	93/200	120/198

[a] Except for the last row of figures, numerator is number of plants infected; denominator, number of plants used.
[b] Numerator is number of aphids on test plants at end of test feeding period; denominator, number of aphids used.

Effect of darkness on the susceptibility of the virus host plant.—It has been demonstrated that keeping test plants in darkness or partial shade for a period of time prior to inoculation (by rubbing solutions of virus over leaves) affects the susceptibility of plants in certain virus-host plant combinations (4,47,49). Limited tests in connection with aphid transmission have failed to yield data indicating an effect of such treatment on transmission (6,56).

This hypothesis was tested in an experiment consisting of 10 replications, with 10 plants per treatment, and a single virus source. A series of 300 lettuce seedlings were arranged on a greenhouse bench in 10 blocks of 3 rows, 10 plants per row. The plants in 1 row in each block were covered with 2-inch stoppered clay pots for 48 hours prior to inoculation. A second row in each block was covered in a similar manner for 24 hours prior to inoculation. The third row in each block was left uncovered and subjected to the normal light and dark cycle of greenhouse conditions (May 25, 1954, weather clear, full sunlight, inoculation done in the afternoon). Just prior to inoculation, the plants were moved into the laboratory and each block was uncovered. The plants were inoculated by means of single green peach aphid apterae or late instar nymphs that had had a preliminary fasting period of 3.91 ± 0.22 hours, an access time on a diseased plant of 4–5 minutes, and a test feeding period of 0.99 ± 0.08 hours. In actual practice a period of 45 minutes was used to make the inoculations, and consequently the periods of darkness prior to inoculation were 48 and 24 hours plus 45 minutes.

The results of the trial were as follows: plants subject to normal greenhouse conditions, 25 of 100 plants infected; 24 hours of darkness prior to inoculation, 40 of 100 plants infected; and 48 hours of darkness prior to inoculation, 48 of 99 plants infected. When corrected for aphids not on test plants at the end of the test feeding period, the results were as follows: normal greenhouse conditions, 22 of 61 plants infected; 24 hours of darkness, 40 of 89 plants infected; 48 hours of darkness, 46 of 87 plants infected. For 48 hours of darkness compared with normal exposure, $\chi^2 = 4.0$, $P = <0.05$.

It was evident that there was less tendency for insects to move off plants that had been subjected to a period of darkness prior to inoculation. Apparently the changes which had occurred within the plant rendered the plant more suitable for aphid feeding. Also, evidence was obtained, in addition to this effect, that the dark treatments increased the susceptibility of the plants to infection by the virus following inoculation by the insects. It would appear that the question is not settled and more data are needed with other vector-virus combinations.

Effect on transmission of pretreating the virus source plant with a period of cold.—Various factors such as the particular virus-plant combination (19), the season of the year (61), the age of infection (56), the stage of plant growth (12), or the tissue region fed on (7) may affect the availability of virus to aphids.

One of the many possible treatments to which the virus source plant can be subjected, as it is under field conditions, is variation in temperature. The following experiment, consisting of a series of 3 replications with 50 plants per treatment and 3 treatments with 3 virus source plants in a Latin square design, was conducted to determine whether or not a short period at low temperature affects the availability of virus to aphids.

Three plants infected with lettuce mosaic virus, all of which had been inoculated by single aphids on the same day and all of which showed similar symptoms were selected as virus sources. The virus source plants were subjected to 1 of 3 treatments: 12 hours in the refrigerator (darkness and cold), 12 hours in the dark at room temperature, or 12 hours under normal greenhouse conditions. The schedule of use of the plants as virus sources in the order given was as follows: morning, evening, and morning. Single green peach aphid apterae or late instar nymphs which had been reared on sugar beet were given a preliminary fasting period of 1.2 ± 0.17 hours and a 4- to 5-minute access time on the treated virus source plants before being transferred to test plants for a test feeding of 1.02 ± 0.12 hours.

Infections in tests with the different source plants were as follows: 12 hours at 5–6°C prior to use, 36 of 150 plants infected; in the dark at room temperature (22–33°C) for 12 hours, 45 of 150 plants infected; and normal greenhouse conditions for 12 hours, 44 of 150 plants infected. No significant differences were found among treatments applied to the virus source plants. The results tabulated on the basis of virus source plant were as follows: source A, 50 of 150 plants infected; source B, 35 of 150 plants infected; source C, 44 of 150 plants infected. The variation due to the particular source plant was of greater consequence than that due to treatment. When only the data from plants which had aphids on them at the end of the test feeding period were used, the results with the various treatments, virus sources, and replications were as follows: cold and darkness, 32.9 per cent (26/79) infection; darkness at room temperature, 37.2 per cent (38/102) infection; normal greenhouse exposure, 37.2 per cent (28/71) infection; source A, 41.5 per cent (37/89) infection; source B, 30.8 per cent (24/78) infection; source C, 36.5 per cent (31/85) infection; replication I, 42.2 per cent (35/83) infection; replication II, 40.8 per cent (36/88) infection; and replication III, 25.9 per cent (21/81) infection. The treatment variation was least ($\chi^2 = 0.484$, $P = <0.50$), the virus source variation was of more consequence (adjusted $\chi^2 = 1.765$, $P = <0.20$ comparing sources A and B), but the variation due to replication was most significant (adjusted $\chi^2 = 4.125$, $P = <0.05$, comparing replications I and II).

The same leaf was used to feed the aphids for 3 consecutive days. Whether or not the feeding activities

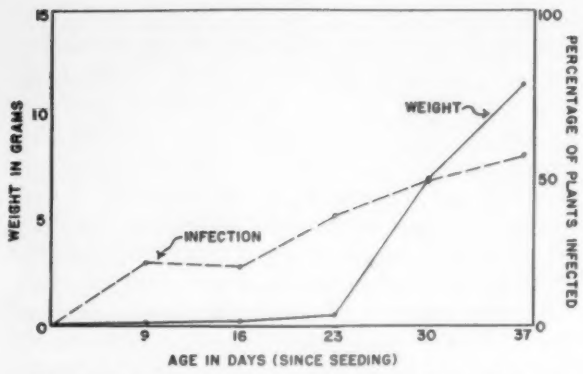

FIG. 1. Variation in weight of lettuce plants of 5 ages and the percentage of such plants infected by lettuce mosaic virus following uniform inoculation by means of aphids.

of the aphids affected the virus availability is not known, but in view of the hypothesis that has been presented by Kirkpatrick and Ross (35) that feeding activities of aphids may reduce plant susceptibility, it should be borne in mind that direct aphid injury might also affect the virus source plants.

Effect of age of test plants on susceptibility.—There are few reports of tests on the influence of age on susceptibility of host plants of nonpersistent aphid-borne viruses. With the Brassica nigra virus it was found that mustard plants from 1–6 weeks in age were approximately equal in susceptibility (56). The following experiment was done to determine the susceptibility of lettuce plants varying in age from 1 to 5 weeks (plus 2 days) in age.

Each week for 5 weeks lettuce was planted in a sterilized seedpan. One week after planting, the seedlings were transplanted. Two days after transplanting the seedlings from the fifth seedpan, all plants were inoculated. The plants were arranged on a greenhouse bench in randomized blocks of 4 replications and 5 treatments, with 20 plants per treatment. Inoculation was made by means of single green peach aphid apterae or late instar nymphs which had been reared on sugar beet and given a preliminary fasting of 4.7±0.45 hours, a 4-to 5-minute access time on a virus source, and a test feeding period of 3.0±0.4 hours. A different virus source was used for each replication. The 4 virus source plants were single-aphid subinoculations from 1 infected plant. Because of the difficulty of locating aphids on the larger plants, the test feeding period was ended by fumigation.

The following are the results: plants 9 days old from seeding, 24 per cent (19/79) infection; 16 days old, 18.8 per cent (15/80) infection; 23 days old, 35.6 per cent (26/73) infection; 30 days old, 40 per cent (36/75) infection; and 37 days old, 54.5 per cent (43/79) infection. The results of the replications were as follows: I, 44.3 per cent (43/97) infection; II, 33 per cent (32/97) infection; III, 30.3 per cent (30/99) infection; and IV, 36.5 per cent (34/93) infection.

After the first 2 weeks the trend was for the plants to increase gradually in susceptibility (Fig. 1), and the variation due to age was greater than that due to replication (virus sources). However, the factor of restlessness of the vector must be considered, for as the plants became larger (because of age) there was less chance that the insects would wander off of them during the test feeding period. Thus the results could be explained on the basis of insect feeding, and further tests would have to be made before it could be determined whether or not susceptibility of Great Lakes lettuce plants to infection with aphid-borne lettuce mosaic virus increases with age.

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FUNCTIONAL ROLE OF COMPATIBILITY FACTORS AND AN IN VITRO TEST FOR SEXUAL COMPATIBILITY WITH HAPLOID LINES OF USTILAGO ZEAE [1]

J. B. Rowell

SUMMARY

Functional roles of the compatibility factors for sex in haploid lines of *Ustilago zeae* (Beckm.) Ung. were identified by observations of the behavior between individual sporidia paired by micromanipulation on drops of dilute corn-coleoptile-extract agar. Under these conditions sporidial fusions were observed in compatible combinations within 3–4 hours after mating and a distinct hypha was produced within 3–4 hours after fusion. That such hyphae were dicaryotic was indicated by the reisolation of both parental lines from cultures of single hyphal tips.

The tetrapolar sex groups of *U. zeae* established by corn inoculation studies were postulated to result from the segregation of 2 pairs of factors, one with 2 (a^1 and a^2) and the other with multiple alleles (b^1, b^2, b^3, . . . b^n). Fusions were observed between mated sporidia of all a^1 and a^2 lines paired, but never between sporidia of lines having similar *a* alleles. The *a* factors apparently govern sporidial fusion. Infection hyphae that developed from fused sporidia having different *b* alleles (example, $a^1b^2 \times a^2b^3$) were straight, grew rapidly, branched rarely, and produced aerial haploid conidia only after approximately 24 hours' development. The hyphae produced by fused sporidia having similar *b* alleles (example, $a^1b^2 \times a^2b^2$) were sinuous, grew slowly, branched frequently, and produced aerial haploid conidia within 12 hours. Thus, the *b* alleles govern the vigor and stability of the dicaryon and apparently the compatibility of the paired nuclei.

The observations on the vigor of hyphae produced by paired sporidia were substantiated with matings of sporidial mixtures on corn-coleoptile-extract agar. The production of infection hyphae by compatible lines under these conditions was a useful quick test for identifying the compatibility factors among 669 monosporidial isolates from 168 sori collected from the field. Biochemical mutants and other lines cultured on agar media for long periods of time, however, produced few or no infection hyphae in tests with compatible lines. The results indicated that infection of inoculated corn seedlings was a more reliable index of sexual compatibility between haploid lines than was the production of infection hyphae on corn-coleoptile-extract agar.

The existence of multiple sex groups among haploid lines of *Ustilago zeae* (Beckm.) Ung. has been established by the corn inoculation experiments of various investigators (3, 4, 11). Recent studies have shown that the compatibility of these sex groups results from the segregation of 2 pairs of factors, one with 2 and the other with multiple alleles (9). Although these factors are essential for the production of galls and chlamydospores in corn, such inoculation tests do not distinguish between sexual and pathogenic functions. The object of the present investigation was to determine if both pairs of factors affected the compatibility of mated sporidia *in vitro*. The results, parts of which have been published (6), indicated that these factors have a functional role in the processes of sporidial fusion and development of the dicaryon.

EXPERIMENTAL METHOD.—Although the occurrence of fusions in mixtures of compatible sporidia of *U. zeae* has been reported by Sleumer (10) and Bowman (2), similar tests at Minnesota were of indifferent success and the actual fused sporidia were difficult to dis-

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tinguish from certain phases of vegetative growth. Therefore, a more reliable technique was required to determine the reaction between 2 individual sporidia of compatible lines. Winge and Lausten (12) paired individual cells with a micromanipulator to determine the sexual reaction between ascospores of yeast, and Holton (5) used a similar method with the secondary sporidia of *Tilletia* spp. This method was adapted for use with *U. zeae*.

Preliminary tests with various nutritive agars indicated that fusions occurred most abundantly and rapidly on a medium containing a dilute extract of coleoptiles cut from seedlings of corn 4–5 days old. Twenty g of fresh coleoptiles were extracted in 80 ml of distilled water by fragmentation in a Waring Blendor. The extract was sterilized by Seitz filtration, and 5 ml added per liter of sterile molten water agar (16 g Bacto Nobel's agar/l distilled water) cooled to 50°C. The 2 haploid lines were tested on hanging drops of this agar by seeding 1 side of the drop with 1 line and the opposite side with the second line. After 24 hours' growth a single young sporidium from 1 line was moved to the center of the drop with a micromanipulator. A second sporidium was then moved from the other line to the center until it was touching the first sporidium. The drops were then incubated at approximately 27°C and observed at regular intervals. Under these conditions direct fusion occurred between many pairs of compatible sporidia within 3–4 hours and a distinct hypha was produced 3–4 hours after fusion.

Results.—*Behavior of paired sporidia.*—Six monosporidial haploid lines with different combinations of *a* and *b* alleles were selected for tests of sporidial fusions and the cells were mated in all possible combinations by the technique outlined. Each mating was tested by the reaction between 8 pairs of cells. All matings were duplicated in 2 trials. Fusion occurred readily between compatible sporidia; in all, 64 per cent of the mated compatible sporidia fused. The range for individual matings was from 100 per cent to 13 per cent of the sporidial pairs for the lines, NyCd × OhBd and OhAf × NyAd, respectively.

Fusion was not observed in any test between lines having similar *a* alleles. However, sporidial fusions were observed in all combinations of sporidia that mated the alleles a^1 and a^2, regardless of the *b* allele present. Furthermore, there was no appreciable difference in the ease of sporidial fusion between lines having similar or dissimilar *b* factors, the percentages of fused pairs for the respective combinations being 63 and 65.

Two distinct types of hyphae developed from fused sporidia. The hyphae produced by fused sporidia from lines of different *b* alleles were straight, grew rapidly, branched rarely, and produced aerial chains of conidia only after approximately 24 hours' growth. These were apparently dicaryotic infection hyphae and were comparable to the vigorous hyphae (suchfaden) Bauch (1) observed in comparable matings of sporidia on weakly alkaline malt agar. Figure 1, A illustrates an infection hypha produced by such a combination 6 hours after mating the sporidia. Hyphae which developed from fused sporidia having the same *b* alleles were sinuous, grew slowly, branched frequently, and produced aerial chains of conidia within 12 hours. An example of this type of hypha 6 hours after mating is illustrated in Figure 1, B. The fusion of sporidia in certain matings of lines nonpathogenic to corn was observed by Sleumer (10) and called "Wirrkopulation." Similarly, Bauch (1) observed that some nonpathogenic matings produced atypical hyphae in his tests which he designated as "Wirrfaden." Presumably, "Wirrkopulation" and "Wirrfaden" are the same as the sporidial fusions and the resulting weak dicaryotic hyphae produced by matings between sporidia of dissimilar *a* and similar *b* alleles in the present study.

The presence of both parental nuclei in the hyphae produced by fused sporidia was established by cutting and isolating single hyphal tips with a micromanipulator. When isolated hyphal tips were transferred to potato-dextrose-broth and shake cultured, dissociation to haploid sporidia resulted. Both parental cultural types were recovered among the colonies obtained in dilution plates from broth cultures of isolated tips, and the 2 parental types of colonies were found to be identical in compatibility to each of the respective parents when inoculated into corn in pathogenicity tests. Furthermore, from each of 3 dicaryotic hyphae, the 2 haploid parents were isolated with a micromanipulator from the aerial chains of conidia.

Functional role of compatibility factors.—The 2 factors governing compatibility relationships among haploid lines of *U. zeae* were postulated from the behavior of matings in inoculated corn (9). Previous studies (7) of the monosporidial progenies from chlamydospores established the inheritance of the parental sex factors in each of 6 compatible combinations among 6 representative haploid lines of *U. zeae* tested. Table 1 summarizes the compatibility by 3 criteria (sporidial fusion, dicaryon development, and corn inoculation) of all possible matings.

No evidence was found of compatibility by any characteristic in matings between lines having similar *a* alleles, regardless of the kinds of *b* factors mated. Matings between lines of different *a* alleles had different compatibility, depending on the criteria used. In inoculated corn, galls and chlamydospores were produced only in matings in which different *a* and *b* alleles were combined. Yet paired sporidia of lines with dissimilar *a*'s and similar *b*'s, which were nonpathogenic to corn, fused as readily as those of pathogenic combinations. When the dicaryotic hyphae produced by the different matings were examined, they were found to be vigorous when the 2 *b* alleles were different, but weak and unstable with combinations of 2 similar *b* alleles. Thus, it was concluded that the functional roles for the 2 compatibility factors were as follows: the *a* locus governed the process of sporid-

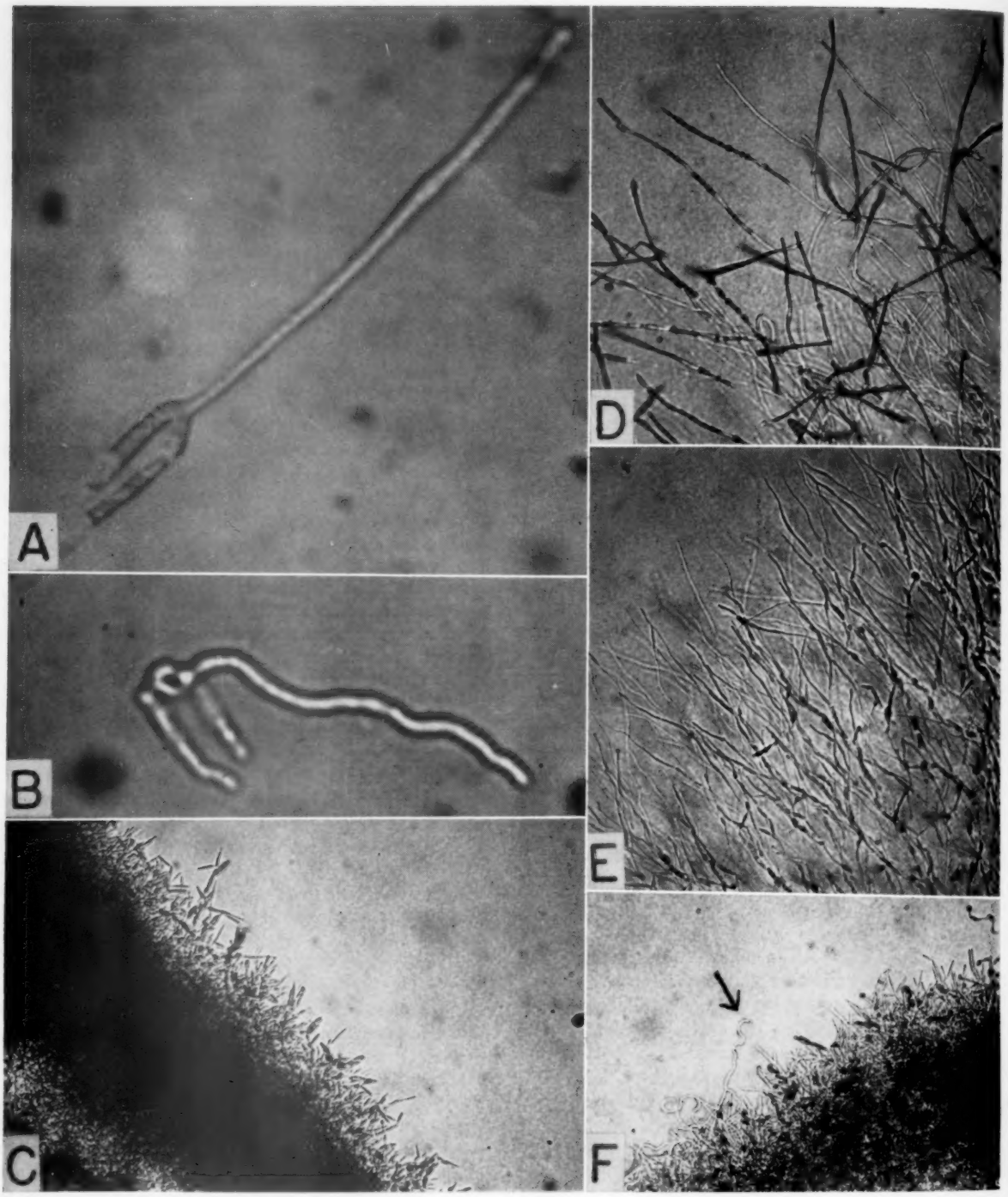

FIG. 1. A and B show character of hyphae produced by fused sporidia 6 hours after sporidial fusion. A) Vigorous infection hypha from fused sporidia of monosporidial lines NyCe (a^1b^4) × OhAf (a^2b^2). B) Weak dicaryotic hypha from fused sporidia of monosporidial lines NyCe (a^1b^4) × NyCd (a^2b^4). Note sinuous form. (Approximately 1600×). C to F illustrate the appearance of mated haploid lines after 24 hours on dilute corn-coleoptile-extract agar. C) Absence of hyphal formation in mixed sporidia of lines OhBd (a^1b^3) × NyCe (a^1b^4). D) Abundant dicaryotic hyphae from mixed sporidia of lines NyCe (a^1b^4) × OhAf (a^2b^2). E) Vegetative hyphae from sporidia of line OhAf which produces mycelial growth under conditions of low nutrition. F) Weak dicaryotic hyphae developing from mixed sporidia of lines NyCe (a^1b^4) × NyCd (a^2b^4) visible only at the margin of the cell mixture (see arrow). (Approximately 240×).

TABLE 1.—*Role of compatibility factors in sporidial fusion, dicaryon development, and chlamydospore formation in inoculated corn for matings among 6 representative lines of Ustilago zeae*

Lines	Compatibility factors	OhAf (a^2b^2)			OhCk (a^2b^3)			NyCd (a^2b^4)			NyAd (a^1b^2)			OhBd (a^1b^3)			NyCe (a^1b^4)		
		F	D	C[a]	F	D	C	F	D	C	F	D	C	F	D	C	F	D	C
OhAf	a^2b^2	−	−	−[b]	−	−	−	−	−	−	+	W	−	+	+	+	+	+	+
OhCk	a^2b^3	−	−	−	−	−	−	−	−	−	+	+	+	+	W	−	+	+	+
NyCd	a^2b^4	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	W	−
NyAd	a^1b^2	+	W	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
OhBd	a^1b^3	+	+	+	+	W	−	+	+	+	−	−	−	−	−	−	−	−	−
NyCe	a^1b^4	+	+	+	+	+	+	+	W	−	−	−	−	−	−	−	−	−	−

[a] F = fusion of paired sporidia; D = character of dicaryotic hyphae produced by fused sporidia; and C = galls and chlamydospores in inoculated corn.
[b] − = none; + = positive and vigorous; and W = weak

ial fusion, and the *b* allele affected the vigor and stability of the dicaryon and apparently the compatibility of the paired nuclei.

Infection hyphae as a test of compatibility.—The production of infection hyphae by mixtures of sporidia on dilute coleoptile-extract agar in Petri dishes was studied as a possible rapid test for determining compatibility. For this test, sporidia from actively growing shake cultures were diluted 1:10 in sterile distilled water. Drops of the suspensions for the appropriate lines were mixed with each other on the surface of agar, and were examined microscopically after incubating for 24 hours at 26–27°C. The 6 representative lines of the previous study were mated in this manner and the compatible combinations were readily distinguished by the characteristic infection hyphae.

With lines that grow as sporidia under these conditions, no hyphae were apparent after 24 hours in tests of single lines, or in matings of lines with similar *a* alleles (Fig. 1, C). The differences in hyphae developing from fused sporidia combining similar and dissimilar *b* alleles were distinct under these conditions. Combinations of lines with dissimilar *a* and b alleles have a profusion of long, straight, rarely branched, aerial hyphae (Fig. 1, D). Isolations of the tips of these hyphae in the manner previously outlined yielded both haploid parents and indicated the dicaryotic nature of the hypha. The aerial habit of these hyphae, with the consequent difference in light refraction, distinguished them from vegetative hyphae produced by mycelial haploid lines which generally are in or on the surface of the agar medium (Fig. 1, E). The hyphae produced by fused sporidia of lines with dissimilar *a* but similar *b* alleles lack the vegetative vigor of the other 2 types of hyphae (Fig. 1, F) and the majority of the hyphae have dissociated to the sporidial condition after 24 hours. However, the few remaining dicaryotic hyphae have the characteristic short, sinuous form. In combinations of a^1 and a^2 haploid lines with similar *b* factors, weak dicaryotic hyphae could not be detected if 1 of the lines produced vegetative hyphae. The characteristic infection hyphae produced by compatible matings pathogenic to corn, and the weak dicaryotic hyphae present in matings of sporidia of dissimilar *a* and similar *b* alleles substantiated the behavior observed in matings between individual sporidia.

A group of 8 lines of known compatibility factors were then selected for matings to test the validity of the method as an *in vitro* test of sexual compatibility. An assistant[2] mated these lines in all possible combinations under a randomized code. All 13 of the compatible combinations among the 36 matings were accurately recognized by the characteristic infection hyphae. One incompatible combination (OhAf × OhCk) produced a profusion of vegetative hyphae and was scored as a doubtful compatible mating. The test was repeated under a second random plan with almost identical results including the inaccurate scoring of the same incompatible combination (OhAf × OhCk) as having the doubtful presence of infection hyphae. However, in this trial 1 compatible combination was recorded as a doubtful incompatible mating because few infection hyphae were present.

During these tests, the mated sporidia were examined microscopically at 4-, 8-, 14-, 24-, and 48-hour intervals. In 6 of the 13 compatible combinations, fused sporidia were found after 4 hours, and in 10 after 8 hours. At the latter time, infection hyphae were apparent that were easily traced to the point of origin from fused sporidia. Sporidial fusions were also found in 2 of the 3 combinations mating similar *b* factors. After 14 hours' development the fused sporidia were impossible to distinguish. This was due partly to the fact that the cytoplasmic contents of the fused sporidia appear to concentrate in the tips of the developing dicaryotic hyphae and the transparent and apparently empty parent cells become difficult to detect. The infection hyphae were most distinct at 14 and 24 hours after mating. Examination after 48 hours required careful scrutiny to differentiate infection hyphae among the abundant vegetative hyphae and aerial chains of conidia present at that time.

The compatible combinations among these matings could not be accurately scored by direct visual examination. Under these conditions, each line alone had a characteristic habit in which various amounts of aerial

[2] Mrs. Elsa Jackson Wood.

conidia and vegetative hyphae were produced. Furthermore, other lines produced few infection hyphae when mated with compatible lines. Thus, the macroscopic criteria of whitish overgrowths or of mycelial halos around the cell masses were frequently inaccurate and microscopic examination for infection hyphae was the most reliable criterion of compatibility.

The accuracy of tests for infection hyphae as a criterion of sexual compatibility was investigated further during a study of the distribution of the *b* factors in natural infections (7). All matings for identifying the compatibility factors of monosporidial lines from 168 sori collected from naturally infected field corn were tested for the production of infection hyphae on coleoptile-extract-agar and for chlamydospore formation in inoculated corn, by the partial vacuum inoculation method of Rowell and DeVay (8). In the preliminary phases of this study, 1645 matings involving 669 monosporidial lines were made, and in only 2.1 per cent of the tests did the 2 methods disagree. Retests of these matings showed that of these inconsistencies, 10 were compatible combinations in which infection hyphae were so sparse as to escape detection, and 24 were incompatible combinations with abundant vegetative mycelia that were erroneously scored as possible infection hyphae. In the final tests of this study, 5002 matings among 441 monosporidial lines had only 1.9 per cent erroneous designations. Of these, 80 were compatible combinations in which the infection hyphae were overlooked, while the remaining 16 errors were incompatible combinations in which vegetative mycelia were scored inaccurately as infection hyphae. Solopathogens were also detected by this test and 12 were found among the monosporidial isolates of this study. Such lines produced characteristic infection hyphae on coleoptile-extract-agar, although these were shorter and less vigorous than those produced by matings of compatible haploid lines.

These tests indicated the usefulness of the formation of infection hyphae on coleoptile-extract-agar as a quick test of compatibility between isolates from wild materials. However, indifferent results were obtained in tests with many lines with a long history of cultivation on artificial media. For example, lines 10A4 and 17D4, which have been in culture at Minnesota for 20 years, produced few or no infection hyphae in combinations with many compatible lines. Yet these same combinations readily produced galls and chlamydospores in inoculated corn. Another line, GuCCa, has never produced infection hyphae with any of numerous compatible lines with which it was mated; thus, in matings with 109 lines with which galls were formed in corn, there were no infection hyphae formed on coleoptile-extract-agar. A retest was made of the compatibility factors of 88 of the monosporidial lines used in the epidemiology study after these cultures had been stored on potato-sucrose-agar for 6 months. Again the results of matings on dilute coleoptile-extract-agar and in corn seedlings were compared. The results of the corn inoculation tests were the same as the earlier tests and indicated the compatibility factors were unchanged. However, 16 per cent of the 277 matings on agar medium were incorrect. A collection of 16 biochemically deficient lines[3] also failed to produce infection hyphae in tests with compatible lines although the original wild parent lines from which these were derived had distinct positive reactions. Thus, the accuracy and usefulness of tests on coleoptile-extract-agar were influenced by the history of laboratory culture of the lines.

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ETIOLOGY OF LETTUCE ANTHRACNOSE AND HOST RANGE OF THE PATHOGEN[1]

Houston B. Couch[2] and R. G. Grogan

SUMMARY

Detached conidia of *Marssonina panattoniana* (Berl.) Magn. survived for 7 days on lettuce seed, and for 9 days in soil held at its moisture equivalent. Uncleaned seed harvested from plants that had been heavily infected in the early stages of plant growth was free of contamination, while over 90 per cent of the volunteer plants from the same seed source that were growing in the parent field were infected.

Anthracnose developed on plants grown in unscreened soil samples taken from a commercial lettuce field 7 months after the last active stages of the disease were evident. Six months after the disappearance of visible signs of the pathogen, isolations from healed lesions on old wild lettuce plants yielded cultures of *M. panattoniana.*

Infection occurred when inoculated plants were exposed to a saturated atmosphere for as short a time as 2 hours. Conidia were not detached when sporulating lesions were exposed to a dry wind, but when a moist air stream was passed over wetted lesions, the spores were detached and moved in the wind current.

Infection of the host occurred both by direct penetration and through open stomates, with the latter mode being more common. Once infection was established, the fungus ramified through the host tissue both intercellularly and intracellularly.

Thirty-eight other species, representing some 35 genera in the Compositae, were tested as possible hosts. Of these, only *Lactuca* spp. and *Bellis perennis* (English daisy) were susceptible.

Lettuce anthracnose, caused by *Marssonina panattoniana* (Berl.) Magn., occurs on commercial and wild lettuce in California during the cool wet months of February and March, but becomes inactive during the warm dry summer months. The disease recurs regularly each year in the Sacramento Valley but is not usually of major economic importance. Occasionally it has caused considerable damage to the spring crop in the Sacramento and Salinas valleys (5).

Because conditions are unfavorable for development and spread of the disease in most parts of California for at least 6 months of the year, the question arises as to how the pathogen survives from 1 season to the next. A review of the literature shows a divergence of opinion concerning the mode of carryover of the pathogen. Although the most comprehensive work on the subject to date favors carryover as contamination of the seed surface (10), other workers (3, 7, 8) indicate that the probable source of primary inoculum is infested debris from the previous crop. The present work was undertaken to study anthracnose of lettuce under California conditions, with particular emphasis on the source of primary inoculum and other etiological factors which might influence occurrence, development, and spread of the disease.

MEANS BY WHICH THE FUNGUS SURVIVES THE DRY SEASON.—*Survival as free conidia on the seed.*—Pape (8) noted infection in the seed-bearing portion of lettuce plants, but found that spores were not viable when taken from infected material that had been dried in a warm room for 7 months. Brandes (3) reported that free conidia were able to survive for only 5 days when held on dry slides. He theorized that the fungus might be carried on the seed, but did not offer experimental evidence in support of this. Stevenson (10) concluded that the pathogen is seed-borne and suggested that lettuce seed could become contaminated with dislodged conidia from infected plant parts at the time of threshing. She further reasoned that the fungus had not been isolated from seed because the fungus grows so slowly in culture that it becomes overgrown by other seed-borne organisms. Successful isolation, then, would depend on the ability to separate *M. panattoniana* from other organisms associated with the seed.

In 1902, Bolley (2) developed a technique for determining the presence of spores of the wheat bunt organism, *Tilletia foetida* (Wallr.) Liro, and of the flax wilt fungus, *Fusarium oxysporum* f. *lini* (Bolley) Snyder & Hansen, on seed of their respective hosts. This consisted of washing suspected seed with distilled water, centrifuging the wash water in order to accelerate the rate of sedimentation, and making a microscopic examination of the sediment for the spores of the organism in question. The length of centrifugation required was not indicated. In the present investigation, however, trials with aqueous spore suspensions of *M. panattoniana* showed that a centrifugation of 8,000 rpm for 15 minutes was sufficient to throw down over 90 per cent of the conidia.

A 100-g sample of lettuce seed was sprayed with a conidial suspension, allowed to air dry, and stored at 21°C. After 3 weeks, this seed was washed with 200 ml of sterile distilled water, the wash water was centrifuged, and only enough supernatant was retained in the centrifuge cup to suspend the sediment. A microscopic examination of the concentrate showed that conidia of *M. panattoniana* were present, and that the percentage of recovery was high. This concentrated spore suspension was sprayed on healthy lettuce plants of the variety Bibb, and these plants, along with checks

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that had been sprayed with a fresh spore suspension, were placed under conditions of 100 per cent relative humidity at 17°C. Ten days later, the check plants showed typical disease symptoms, but the plants that had been sprayed with the concentrate remained symptomless. The conidia apparently had not survived the 3-week period of drying. A second trial was conducted in which lettuce seeds were sprayed with a spore suspension of *M. panattoniana*, dried as in the previous experiment, and samples taken at 0, 2, 4, 8, and 16 days. Infection was obtained from washings of samples taken immediately, and on the second and fourth days, but the disease did not develop on plants sprayed with concentrate from the samples dried 8 and 16 days. In a third, and similar experiment, samples were taken at daily intervals for 10 days. In this trial, infection resulted from samples taken up to and including the seventh day, but those taken thereafter gave negative results, although their checks were severely diseased.

Survival in the debris of previously infected plants.—Brandes (3) reported that the fungus could survive for 3 months in diseased lettuce leaves packed in cans filled with sand, and suggested that a common source of greenhouse infection is contaminated debris left to overwinter in the soil. Newhall (7) found that soil beneath diseased lettuce plants harbored the fungus for a period of 3 months, but was unable to detect its presence after 6 months. He suggested that the organism probably overwinters in New York in native *Lactuca* spp., particularly *L. serriola* var. *integrata*. Stevenson (10) stated that the lettuce anthracnose fungus may be harbored in infested trash for 8 months, and that this period may be longer under certain local conditions. However, she minimized the importance of this type of carryover, and suggested that the phenomenon of yearly appearance of anthracnose in the same spots might have been due to the soil and atmospheric peculiarities of these areas that would have made them particularly favorable for the development of the disease after the fungus had been introduced with the seed.

Almost all of the plants in a 5-acre field of lettuce grown for seed near Clarksburg, California, showed anthracnose in 1952. Irrigation in this field was by overhead sprinkling. This, combined with a cool spring and early summer, accounted for the presence of active lesions on the basal leaves up to the middle of July. In no case, however, was the disease found in the inflorescences which developed later in the summer. On February 10, 1953, soil samples were taken from areas of this field that were free of volunteer plants. Half of this soil was sifted through a fine screen and carefully examined in order to exclude all readily visible pieces of plant debris. The other portion, which contained a generous amount of plant refuse from the preceding crop, was used as it came from the field. The soil samples were placed in separate groups of flats and planted 1 week after collection with seed of the lettuce variety Imperial 152 that had been treated for 10 minutes with sodium hypochlorite (5000 ppm active Cl). After emergence, both groups of flats were placed in a continual mist, and a fine stream of water was used periodically to splash soil on the growing plants. Five weeks after planting, several infected plants were found in the flats which contained the unscreened soil. When the experiment was discontinued 2 weeks later no infection was found on plants in any of the flats that contained the screened soil.

On April 23, 1953, similar samples were taken from the same field and treated in the manner previously outlined. The amount of debris in the unscreened group in this case, however, was very small, because of decomposition which had been accelerated by a thorough disking about 1 month after the first sample was taken. These plants were held for 2 months, and although the inoculated checks showed anthracnose, no disease developed in either of the 2 groups.

In February 1953, this field was divided into 36 equal blocks, and 100 volunteer lettuce plants checked at random in each block for anthracnose. This count showed that 93 per cent of the plants in the area originally occupied by lettuce in 1952 were diseased and that the degree of infection was fairly uniform throughout. The land which formed the eastern boundary of this field had been planted to onions in 1952, but contained a large number of volunteer lettuce plants from seed that had blown over from the adjacent lettuce field the previous year. Examination of the onion field showed a gradation in percentage infection from the border next to the old lettuce planting to an average of 40 ft. into the field. From this point on, no anthracnose was found in the volunteer lettuce plants.

An attempt was made to wash spores from a 2-lb. sample of uncleansed seed harvested from the 1952 crop in the diseased field. The centrifugation technique previously described was used. No spores of *M. panattoniana* were found in the concentrate and the disease did not develop on plants sprayed with it, although inoculated checks were severely diseased. As a further check on this seed, 18 flats were planted with it, each with approximately 100 seeds. After emergence, the plants were watered by overhead sprinkling and when they were discarded 2 months later, no evidence of anthracnose had been found, although a high disease incidence in check plants inoculated at intervals during the 2-month test period and held under similar conditions, showed that the environment was favorable for disease development.

Survival in previously infected plants that remained alive during the summer months.—Inactive anthracnose lesions on old wild lettuce plants are characteristic in appearance. The spots are usually slightly raised, and have light tan centers surrounded by brown borders. In many cases, these lesions coalesce, blending into large light tan areas with brown margins (Fig. 1). It was in plants such as these that Newhall (7) suspected the organism to overwinter in New York.

On November 14, 1953, stems of *L. serriola* var. *integrata* showing lesions similar to those in Figure 1 were collected from plants that had been growing all summer. The disease had not been active in these plants since late spring. These lesions were peeled off and placed in a Waring Blendor with enough sterile distilled water to suspend them. They were then cut to a fine pulp, care being taken not to allow the mixture to become hot during the process. The material then was filtered through a single thickness of cheesecloth, and the filtrate sprayed on healthy lettuce plants of the variety Bibb. These plants were placed in a humidity chamber and held at 100 per cent relative humidity and 21°C for 24 hours. At the end of this period, they were removed to a room with a temperature range of 12–17°C and a relative humidity of 70 per cent. On November 21, lesions typical of anthracnose were found on some of these plants. The lesions were not numerous, but examination of the associated fungus verified that it was *M. panattoniana*.

Survival of spores in the soil.—It has been postulated that the fungus might survive in the soil as a saprophyte (3). However, insofar as the writers know, no attempt has been made to date to study this possibility.

A sample of field soil was infested with a spore suspension and stored in an air-tight container at 21°C. Samples were taken at 0, 1, 2, 4, 16, and 32 days after the initial treatment, wetted to a pasty consistency, and applied to moistened leaves of healthy lettuce plants. After treament, the plants were placed in a humidity chamber and held at 21°C and 100 per cent relative humidity for 24 hours. At the end of this time they were transferred to a room held at 12–17°C and 70 per cent relative humidity. Sufficient inoculated checks were made at each sampling to ascertain that the environment was suitable for the development of the disease. Infection was evidenced up through the 8-day sample, after which the treated plants remained symptomless, although the inoculated checks became diseased in all cases. From this experiment it appeared that the critical period for survival of spores in the soil was between 8 and 16 days. A second trial was run in which Yolo clay loam, with a moisture equivalent of 22.5 per cent, was used. A portion of the soil was sterilized by autoclaving at 15 lb. pressure for 15 minutes, and the remainder was used as it came from the field. To both was added enough aqueous spore suspension to bring the soil to its moisture equivalent. After treatment, the 2 groups were stored in air-tight containers at 21°C. Beginning with the third day after inoculation and continuing to the sixteenth day, soil samples were taken each day from the 2 groups and applied to healthy leaves in the same manner as described above. In both the sterilized and unsterilized groups, infection was noted up to and including the ninth day, after which the disease did not develop, although the inoculated checks showed abundant infection.

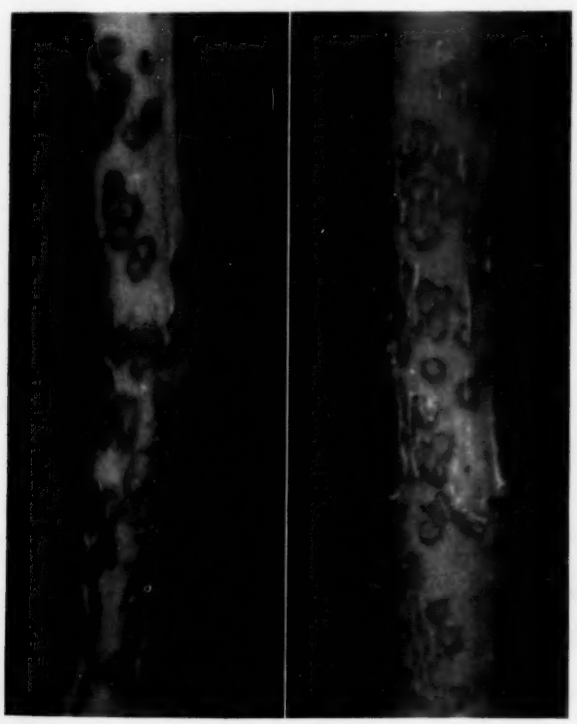

FIG. 1. Inactive anthracnose lesions on stems of wild lettuce (*Lactuca serriola*, var. *integrata*).

LENGTH OF EXPOSURE TO SATURATED ATMOSPHERE REQUIRED FOR PLANTS TO BECOME INFECTED.—Concerning the critical period of free moisture on the plant required for infection to take place, Brandes (3) stated that "The spores of the fungus will probably not germinate and enter into the lettuce leaf when transferred, unless the leaves stay wet for a considerable time—6 to 12 hours." Three experiments were conducted to determine this point. Healthy lettuce plants were sprayed with a spore suspension of *M. panatoniana*, placed in a humidity chamber at 21°C and 100 per cent relative humidity, removed at hourly intervals from 1 to 8 hours, dried in front of an electric fan, and then placed in a room held at 12–17°C and 70 per cent relative humidity. In 2 trials, infection was obtained after a 2-hour exposure to a saturated atmosphere, while in all 3 trials infection occurred after exposure to a saturated atmosphere for 3 hours or longer.

SPREAD OF THE FUNGUS IN NATURE.—Brandes (3) has shown that the fungus may be spread from 1 plant to another by splashing water. Gardner and Kendrick (5) suggested the possibility of spread in the field by severely infected leaves that are easily detached and blown about during periods of heavy winds. Another possible means of spread is wind-borne conidia. However, members of the order Melanconiales are not adapted to the dissemination of conidia by dry air because they are produced in sticky masses on acervuli. Wilson (12), for example, has shown that coni-

dia of *Coryneum beijerinckii* Oud., cause of the shot-hole disease of stone fruits, are only rarely detached by dry air, but after being wetted are easily disconnected in great numbers. The purpose of the following experiment was to attempt to determine if conidia of *M. panattoniana* could be picked up in the wind stream under dry conditions, and if not, whether or not it was possible for them to be disseminated by a wind current after being wetted.

Four lettuce plants heavily infected with *M. panattoniana* were used as the source of spores. An electric fan was used to pass an air current over them and then over 5 healthy lettuce plants placed 3 ft. away. The leaves of the test plants were kept wet throughout the time of exposure to the air passing over the spore-source. In 1 trial the air stream was passed over dry lesions, in another, the lesions of the spore source were kept moist with an atomizer. After these treatments, the exposed plants were placed in a humidity chamber and held at 21°C and 100 per cent relative humidity. Ten days after exposure, abundant anthracnose lesions had developed on plants that had been exposed to the air current passed over wet lesions, but no disease was present on those held in front of the air current passed over dry lesions.

INFECTION AND PROGRESS OF THE FUNGUS WITHIN THE HOST.—Appel and Laibach (1) studied penetration of lettuce leaves by *M. panattoniana* and reported that the fungus punctured the epidermal cells directly. They illustrated penetration at the junction of epidermal cells, and emphasized that they had never observed the fungus to enter through open stomates, although it did penetrate the epidermis near them. Also, they theorized that the fungus was aided in penetration by enzymatic action. This was based on the observation that a brownish discolored area occurred in the epidermal wall surrounding the point of entry of the fungus. Brandes (3) reported that *M. panattoniana* penetrated its host only at the junction of epidermal cells, and, because he never observed the germ tube within a cell, concluded that the mycelium probably continued to grow between the cells thereafter.

In this study, portions of lettuce leaves of the variety Bibb were inoculated with a conidial suspension of *M. panattoniana* and placed on moist filter paper in petri dishes. After 24 hours incubation at 21°C, the leaf tissue was immersed in lacto-phenol clearing solution[3] and held at a temperature just below boiling for 30 minutes. At the outset, a few drops of 0.05 per cent cotton blue in lacto-phenol was added to the solution. Microscopic examination showed that the fungus had penetrated the leaves by growth of the germ tubes through open stomates. After entry through the stomatal apertures had been accomplished, substomatal vesicles were produced (Fig. 2, A). Although growth of the germ tubes was extensive, sometimes exceeding 100 μ in length, direct penetration of the epidermis was not observed in this experiment.

Examination of naturally infected leaves of wild lettuce was made with material cleared and stained as described above. Early stages of infection were quite abundant and in all cases penetration occurred by way of the stomates. Death of single guard cells was evident (Fig. 2, B) and the fungus ramified through the leaf tissue from these points.

Plants of the variety Great Lakes were sprayed with a conidial suspension and held at 21°C and 100 per cent relative humidity for 24 hours. After this exposure, they were removed to a room held at 12–17°C and 70 per cent relative humidity and periodic samples were taken from them. By means of epidermal strippings it was found that infection of the petioles was accomplished by direct penetration of the epidermis, with no evidence of stomatal entry. Appresoria were observed 24 hours after inoculation, but actual penetration was not noted until 36 hours after the spores were placed on the leaves. In preparations in lacto-phenol, it was found that cuticular puncture of the lamina apparently was accomplished by mechanical pressure, with passage through the wall being facilitated by enzymatic action, as described by Appel and Laibach (1). This enzymatic action was evidenced by a zone with an affinity for cotton blue surrounding the point of contact of the appresorium (Fig. 2, C, D). In many cases this zone was extensive, exceeding the diameter of the appresorium 20 times. Penetration was randomized over the surface of the leaf irrespective of point of contact between epidermal cells (Fig. 2, C, D).

Seven days after inoculation, profuse mycelial growth had permeated the leaf tissue, radiating intercellularly and intracellularly from the point of entry, with little or no limitation by the cell walls. Stomatal infection was also followed by intercellular and intracellular invasion. Ultimately, there was a progressive collapse of epidermal cells, beginning at the point of entry of the fungus.

Progress of the fungus within the host also was studied by means of serial sections. Petioles of the variety Great Lakes that had been artificially inoculated and showed typical anthracnose lesions were fixed and killed with Karpechenco's solution, sectioned serially on a rotary microtome, stained with 1 per cent phloxine for 24 hours, and counterstained with 0.3 per cent fast green in clove oil for 20–30 seconds. Microscopic study of this material showed the mycelium to be both intercellular and intracellular throughout the host tissue.

HOST RANGE.—A review of the literature shows the known host range of *M. panattoniana* to be restricted largely to species of the genus *Lactuca*. Brandes (3) reported that he was unable to produce the disease on *Latuca serriola* L., *L. floridana*, and *Cichorium intybus* L. Parker (9), however, obtained infection of *L. ser-*

[3] Formula for the lacto-phenol clearing solution used: 20 ml distilled water, 20 ml carbolic acid, 20 ml lactic acid, and 40 ml glycerin.

riola and observed the disease on this species in nature. Newhall (7) studied species of *Lactuca* as possible hosts, and reported that besides *L. sativa*, *L. serriola* var. *integrata*, *L. spicata*, and *L. canadensis* were hosts of *M. panattoniana*. He also obtained infection of the following species in the seedling stage: *L. altaica*, *L. laciniata*, *L. saligna*, *L. virosa*, *L. indica*, *L. graminifolia*, and *L. murialis*. Pape (8) and Viennot-Bourgin (11) reported endive, *Cichorium endivia* L., to be a host of *M. panattoniana*. In 1935, anthracnose was reported on endive and escarole in Texas (4). However, this is the only record of this species serving as a host of this fungus in the United States. In England, *M. panattoniana* was recorded on *Crepis capillaris* L. (10). The fungus causing the disease on this host, however, would not infect lettuce, although it appeared morphologically similar to the lettuce form.

In this study, plants of 38 species, representing some 35 genera in the Compositae, were inoculated with *M. panattoniana*. All inoculations were made with spore suspensions of the organism, after which the plants were placed under conditions near optimum for disease development. In all cases, adequate inoculated lettuce checks were made to determine whether the inoculum and environment were suitable for disease development. Plants tested as possible hosts were of the following species: *Achillea* sp., *Ageratum houstonianum* Mill., *Agoseris plebeia* Greene, *Arctotis stoechadifolia* Berg., *Bellis perennis* L., *Brachycome iberidifolia* Benth., *Calendula officinalis* L., *Callistephus chinensis* Nees, *Carthamus tinctorius* L., *Catananche caerulea* L., *Centaurea cyanus* L., *C. solstitalis* L., *Chrysanthemum morifolium* Ramat., *Cichorium endivia* L., *C. intybus* L., *Cosmos diversifolius* Otto, *Crepis capillaris* L., *Coreopsis tinctoria* Nutt., *Dahlia pinnata* Cav., *Dimorphotheca aurantiaca hybrida* D. C., *Gaillardia aristata* Pursh, *Gridelia camporum* Greene, *Helianthus annus* L., *Helichrysum bracteatum* Andr., *Hypochoeris radicata* L., *Matricaria suaveolens* (Pursh) Buch., *Parthenium argentatum* Gray, *Piqueria trinervia* Cav., *Scolymus hispanicus* L., *Scorzonera hispanica* L., *Senecio cruentus* D. C., *Sonchus asper* L., *S. oleraceus* L., *Tagetes erecta* L., *Tithonia rotundifolia* Blake, *Xanthium pennsylvanicum* Wallr., *Xanthisma texanum* D. C., and *Zinnia elegans* Jacq. Of these plants, only *Bellis*

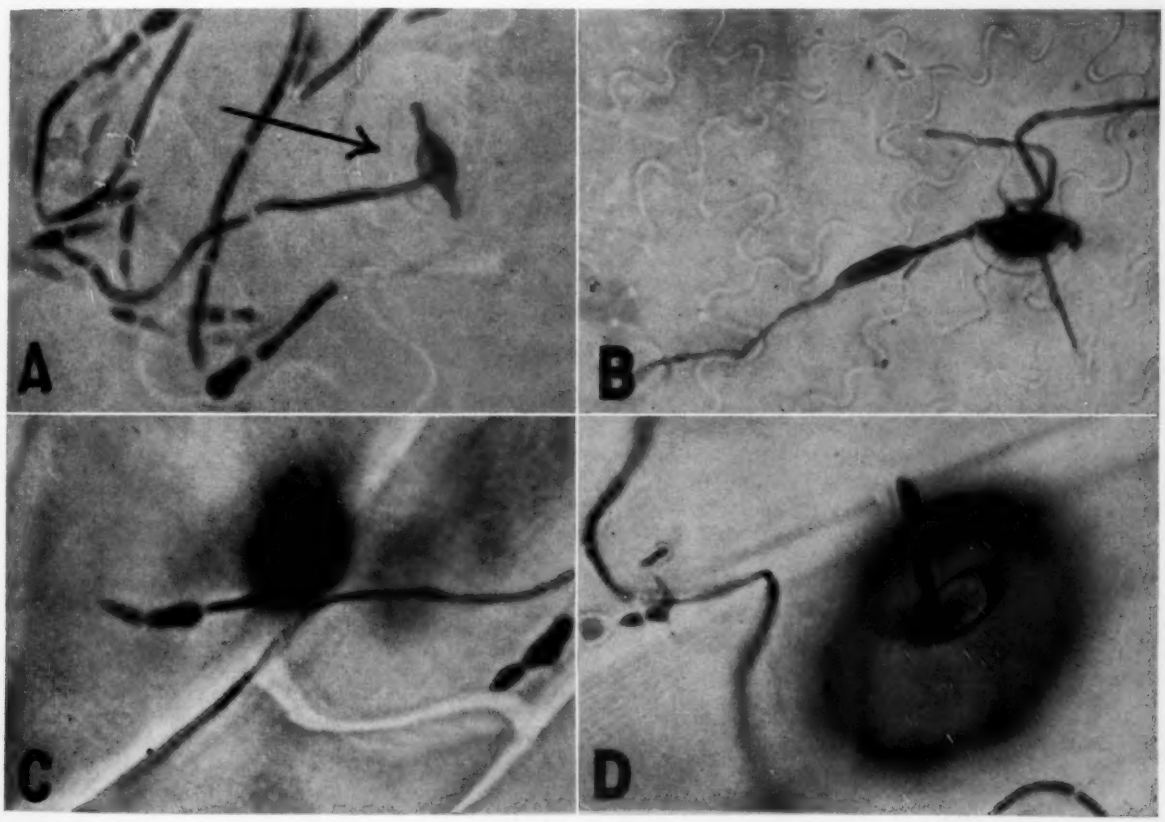

FIG. 2. Mode of host penetration by *Marssonina panattoniana*. A) Stomatal penetration of lettuce leaf. Note substomatal vesicle. 920×. B) Natural infection of wild lettuce leaf, showing stomatal penetration and subsequent death of a guard cell. 860×. C) Direct penetration of epidermis of petiole, showing entry at junction of epidermal cells. 1020×. D) Direct penetration of epidermis of petiole at a point distant from junction of epidermal cells. Note dark stained zone surrounding point of entry. 1020×.

perennis (English daisy) was susceptible. The first evidence of the disease on this species was numerous small sunken spots on the lower surfaces of the leaves. As the disease progressed, these spots enlarged to about 2–3 mm in diameter and became tan in color. In severe infections, the lesions coalesced, giving a necrotic pitted appearance to the lower surface of the leaves. The disease developed to this degree of severity in only a few instances. Sporulation was sparse. As a general rule, the fungus ceased activity soon after the first manifestation of lesion discoloration. *Cichorium endivia*, *C. intybus*, and *Crepis capillaris* did not become infected, although they have been reported as hosts elsewhere (4, 8, 11).

Discussion.—The evidence presented herein shows that *M. panattoniana* is capable of surviving its adverse season in the debris of a previously infected crop. That the fungus is carried over in infested debris and not as a soil saprophyte is borne out by occurrence of the disease only on plants grown in flats of soil containing refuse from a previously infected crop, and by its failure to survive for longer than 9 days as conidia in soil held at its moisture equivalent. Ability of the organism to survive for at least 7 months in infested debris is sufficient to enable it to exist from 1 season to the next under California growing conditions.

The form of the organism in this debris is not known. It is possible that it produces chlamydospores which serve to carry it over its adverse season. Production of these structures in artificial media has been reported (3). To the writers' knowledge, however, these have not been found in the host and none were observed in the present study. There is also the possibility that the spores might be long lived in the matrix on the acervulus. Keitt et al (6) have shown, for example, that longevity of the conidia of *Coccomyces hiemalis* Higgins is reduced considerably when they are washed from the matrix in which they are produced. Pape's report (8) that conidia of *M. panattoniana* were not viable when taken from infected material that had been dried in a warm room for 7 months would not necessarily apply to survival of the organism under field conditions.

It is difficult to reconcile the occurrence of anthracnose in California with the seed-transmission theory of Miss Stevenson (10). In wild lettuce anthracnose recurs consistently every year, yet the disease has never been found in the inflorescences of these plants because the environment becomes unfavorable for its development long before flowering occurs. With commercial lettuce, however, there is a possibility that the seed might become contaminated at the time of harvest. This infestation would occur from dislodged conidia, as suggested by Miss Stevenson (10). However, since free conidia apparently survive for only a few days on lettuce seed, carryover in this manner is highly improbable.

Another possible source of contamination is bits of infested debris mixed with the seed. However, when seed from heavily infected parent plants was planted in sterile soil, and the seedlings grown under environmental conditions favorable for disease development, anthracnose did not develop, whereas volunteer plants from the same seed-source, growing in the seed field the following spring, showed over 90 per cent infection. The absence of contaminated debris in the uncleaned seed sample can be explained by the fact that in California, lettuce seed is commonly harvested by combining, a practice that removes only the inflorescence or seed-bearing portions of the plants. Infection of these plant parts has never been observed, and the possibility of contamination from the infected lower portions of the plants is slight.

In view of the evidence presented herein, it is likely that infested debris of cultivated or wild lettuce in the soil or above ground constitutes the most important, if not the only, source of primary inoculum for lettuce anthracnose under California growing conditions.

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THE ROLE OF ENZYMES IN THE DEVELOPMENT OF FUSARIUM WILT OF TOMATO [1]

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SUMMARY

Filtrates from cultures of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen, grown on moist wheat bran and extracted with water, caused vascular browning and wilt in tomato plants. The active factor in the filtrate was heat labile and nondialyzable, and its action suggested that an enzymatic reaction might be involved. Consequently various commercial enzyme samples were tested on tomato cuttings. Only those enzyme samples with pectic enzyme activity were found to duplicate the action of the culture filtrates. A correlation between the severity of vascular browning and pectin methyl esterase activity was observed in the various commercial enzyme samples. An active pectic enzyme preparation was isolated from the culture filtrates by precipitation with alcohol. This preparation showed pectin methyl esterase activity and depolymerase activiy and produced the typical wilting and vascular browning which are the most specific symptoms in diseased plants. The results indicate that the pectic enzymes produced by the pathogen are important factors in causing the characteristic wilt and vascular browning associated with Fusarium wilt of tomato.

Several theories have been offered to account for wilting of tomato plants (*Lycopersicon esculentum* Mill.) infected with *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen. Among the suggested explanations are various processes of plugging of vessels, e.g. mycelial growth of the pathogen, tyloses, and gums produced either by the host or the pathogen. The toxin theory rests on the assumption that the pathogen, which is confined to the xylem elements, releases into the transpiration stream certain toxic materials that cause wilting of the foliage at points remote from the area of infection. Many workers have shown that filtrates from liquid cultures of the fungus cause wilting of the shoots when the cut stems are inserted in the liquid. The toxin theory was supported by Gäumann and coworkers (9, 10, 11, 26, 27) who isolated a specific toxin, lycomarasmin, from 2- to 4-month-old cultures of the fungus and demonstrated that the substance was toxic to tomato cuttings. Lycomarasmin was reported by Woolley (39) to be a dipeptide of asparagine and a new amino acid.

The typical disease symptoms produced by culture filtrates differ markedly from those produced by lycomarasmin. Gäumann (8) stated that substances in the culture filtrate other than lycomarasmin exert complementary effects. Hodgson and coworkers (17, 18, 19) have shown that various groups of polymers (in 0.1 to 0.4 per cent aqueous solution) can induce wilt in tomato cuttings. With all these polymers a correlation between the severity of wilting and the molecular weight could be demonstrated. It was assumed, therefore, that these substances were toxic because they interfered mechanically with the transpiration system. The action of these polymers differs from that of lycomarasmin, which acts principally on the plasma membrane destroying the necessary conditions for turgor and thus producing wilt. During recent years Scheffer and Walker (28, 29) and Dimond and Waggoner (4, 5, 34) have presented considerable evidence that lycomarasmin is not the chief wilting toxin in tomato plants infected with *Fusarium oxysporum* f. *lycopersici*.

The concept of Gäumann that lycomarasmin is the principal wilting factor does not appear plausible. Most of the experimental evidence can be accounted for on the basis that wilting is due to the plugging of the vascular elements. When Ludwig (23) and Scheffer and Walker (29) applied equal pressure to diseased and healthy stems and petioles, less water moved through the infected vascular system than moved through normal stems. They reasoned that the water shortage caused by an impediment to water movement is the cause of wilting.

The nature of the material causing impediment to water movement in the infected stem is not known, although tyloses from adjacent cells, gums produced either by the host or the pathogen, and even mycelial growth (30) have been suggested. The last named possibility has been investigated by Waggoner and Dimond (35) using a scale model, and they concluded that whereas mycelial growth can contribute to the reduction in flow, it cannot be the total cause of decreased water movement in infected plants.

Scheffer and Walker (29) demonstrated that tomato cuttings treated with 15-day-old culture filtrates exhibit both wilting and vascular browning at the end of 72–96 hours. As these culture filtrates do not contain any appreciable quantities of lycomarasmin, the effects observed were attributable to some other factor or factors present in the culture filtrate. A fuller discussion of the literature pertinent to this paper is published elsewhere (14). The work presented here is an investigation into the nature of this factor or

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factors. A preliminary report has been published (15).

MATERIALS AND METHODS.—Tomato plants of the susceptible variety Bonny Best were grown in the greenhouse in sand and were watered with Hoagland's nutrient solutions. The wilt fungus was grown in 32-oz. pharmaceutical bottles in 100 ml of a modified Richard's medium (37). Cultures were seeded with small pieces of mycelial mat and were grown in still cultures at 28°C. For preparation of replacement culture filtrates the organism was grown on modified Richard's solution for 2 weeks at which time the culture fluid was filtered off and discarded. The mycelial mat was washed in distilled water and suspended in 100 ml of modified Czapek's media (37). After 24 hours, the culture fluid was filtered through a layer of Celite to remove fungus spores. This solution constituted the replacement culture filtrate. Bran culture filtrates were obtained by the procedure of Winstead and Walker (37).

Plant assay.—Assays of preparations for disease-inducing properties were conducted with fresh tomato cuttings averaging about 1 ft. in length. They were placed in the test solutions at 27–28°C and at approximately 50 per cent relative humidity for 2–4 days. The bases of the stems were trimmed once each day and solutions were changed twice daily to reduce the growth of contaminants. On the fourth day the cuttings were rated for both wilt and vascular browning. The severity of wilt was determined on an arbitrary index of 0 to 4; 0 indicating no wilt and 4 indicating maximum wilt. The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2 = browning in the lower $\frac{1}{3}$ of the stem; 3 = browning in the lower $\frac{2}{3}$ of the stem; and 4 = browning from the base to the apex of the stem. Three or 4 plants were used for each assay and the wilting and browning indices were averaged.

Enzyme determinations.—The pectin methyl esterase (PME) and polygalacturonase (PG) activities of filtrates and commercial enzyme preparations were determined by procedures described by Winstead and Walker (37). Pectin methyl esterase activity is expressed as "mg methoxyl groups removed at the end of 3 hours by 1 ml of the culture filtrate or 1 g of the enzyme sample." Polygalacturonase activity is expressed as "units per ml of culture filtrate or g of enzyme powder, a unit being defined as the amount of enzyme required to liberate 0.05 milliequivalent of reducing groups in 5 minutes in a 5-ml aliquot from a reaction mixture containing 0.5 per cent sodium polypectate."

The depolymerase enzyme (DP) differs from PG in that it splits the pectic acid without progressing to the point where monogalacturonic acid molecules are formed. A positive assay by the PG test may be the result of reducing groups freed when the polypectate molecule is split by DP. If no monogalacturonic acid is formed the evidence is strong that DP is concerned and not PG. DP activity was studied by following the reduction in viscosity of the polypectate solution on incubation with the enzyme preparation as well as by running paper chromatograms on the reaction mixture. After incubation of the enzyme with pectin for certain periods of time, aliquots were tested for presence of monogalacturonic acid by means of ascending paper chromatography. The solvent system used was a 4:1:5 mixture of butanol, acetic acid and water and the papers were sprayed with benzidine solution (0.5 g benzidine + 10 ml acetic acid + 10 ml of 45 per cent trichloroacetic acid + 100 ml of 95 per cent ethanol). After spraying, the papers were heated for 2 minutes in an oven at 105°C. The presence of monogalacturonic acid was indicated by a pink spot.

β-Glycosidase activity was followed reductometrically with salicin used as the substrate (33). The procedure adopted was similar to that used in determining polygalacturonase activity. β-Glycosidase activity is expressed in units per g of enzyme powder. A unit is defined as "the amount of enzyme required to liberate 0.04 milliequivalent reducing groups in 5 minutes from a 0.139 M solution of salicin at 30°C."

EXPERIMENTAL RESULTS.—Scheffer and Walker (29) have shown that the filtrate from replacement filtrates lost activity when heated. To determine the degree of heat sensitivity of the active material in the present investigations, suitable quantities of the replacement culture filtrate were subjected to different temperatures for a period of 10 minutes, at the end of which they were assayed on tomato cuttings. The results obtained showed that culture filtrates heated at 60°C for a period of 10 minutes lost their activity. That lycomarasmin was not involved in the expression of disease symptoms is obvious, since Dimond and Waggoner (5) found the half life of lycomarasmin at 63°C to be 560 minutes.

Gäumann (8) has classified wilting toxins into 2 broad groups, namely peptides and quinones. Compounds in both groups have small molecules which should be removed easily by dialysis. Suitable quantities of replacement culture filtrates (about 150 ml) were dialyzed against 1300 ml of water at 4°C for varying periods of time and the activities of the dialyzed liquids on tomato cuttings determined. The results of a typical experiment showed no appreciable loss of activity as a result of dialysis over a period of 24 hours. Compounds with small molecular weights are not responsible, therefore, for the wilting property of the culture filtrate.

Since the active material was heat labile and non-dialyzable, the possibility that it might be a protein was studied next. Suitable quantities of the culture filtrate were incubated with various proteolytic enzymes and maintained at the pH optimum of the respective enzyme for a period of 1 hour at 37°C; the pH then was adjusted to 6.0 and the browning activities of the treated filtrates determined. Suitable controls were

run. Incubation of the culture filtrates with the various proteolytic enzymes definitely lowered the browning activity of the filtrates (Table 1). These results and those from the dialysis and heat experiments indicated that the active material is a protein. The slow action of the culture filtrates coupled with the fact that the Fusaria are known to produce a number of extracellular enzymes led to the supposition that the active protein might be an enzyme.

Various commercial enzyme preparations were next tested at various concentrations to determine whether or not the disease symptoms could be reproduced. Tomato cuttings were exposed to the preparations for 4 days and then indexed for both wilt and vascular browning. Of the 5 enzyme preparations tested, only Pectinol 100 D produced both wilt and vascular browning (Table 2). Some of the others caused wilt but none caused vascular browning. The symptoms of plants treated with Pectinol 100 D resembled closely those of inoculated plants as well as those of plants treated with the culture filtrates.

The source of pectic enzymes in the previous experiment was a commercial preparation and probably was not pure (7, 21). That the effect observed with the commercial enzyme preparation was due to its pectic enzyme activity and not to the presence of other accompanying materials was ascertained in 2 ways. When heated for 10 minutes at 100°C all preparations failed to produce wilting and browning. This fact eliminated the possibility of symptoms being induced by such materials as peptides which might have been present as contaminants. No decrease in activity resulted from dialysis for 24 hours, a further indication that activity is not associated with small molecules. In a preliminary paper (15) it was reported that when tomato plants were treated with several commercial pectic enzyme preparations possessing pectin methyl esterase and polygalacturonase activities, a definite correlation between symptoms produced in tomato cuttings, especially vascular browning, and PME activity was obtained.

Examination of replacement culture filtrates for pectic enzyme activity showed that they contained both PME and PG. The active material from replacement cultures was precipitated with cold alcohol but the precipitate obtained possessed only a part of the activity of the culture filtrate, a considerable amount of the activity having been lost during the precipitation. Since wheat bran has commonly been used as a component of the media in manufacture of commercial pectinases (20), it was tried next. The excellent recovery of the enzymes from bran cultures suggested the use of these cultures for extraction and precipitation of the enzyme. The procedure adopted for the preparation of the enzyme from bran culture filtrates follows. Two hundred ml of water together with a few drops of toluene was added to each of 10 bottles of a 5-day-old culture of Fusarium growing on wheat bran and the cultures were left to autolyse overnight. At the end of 12 hours, the autolysed wheat bran was squeezed through cheesecloth and the extracted liquid was centrifuged in the cold (approximately 4°C) at 6000 rpm for 15 minutes. The clear supernatant was concentrated 3 fold *in vacuo*. Two volumes of cold ethanol was added, the precipitate was allowed to settle for 30 minutes, and the suspension was centrifuged. The precipitate was redissolved in water and lyophilized to give a light yellow fluffy powder. The total yield was 11.6 g. This material was assayed both for PME and PG activity, and its activity tested on plants at 3 different concentrations. This enzyme preparation had a PME activity of 88.7 and a PG activity of 0.202 units/g. When the preparation was tested at concentrations of 0.5, 0.25, and 0.125 per cent on tomato cuttings, wilting indices of 2.8, 2.8, and 2.0 and browning indices of 3.0, 2.2, and 1.2 were obtained. A 0.125 per cent solution of the enzyme

TABLE 1.—*Browning activity of replacement culture filtrates following treatment with proteolytic enzymes*

Expt. no.	Solution tested	Browning index[b]
1	Culture filtrate only	3.0
	1 Per cent papain[a] only	0.3
	Culture filtrate + papain[a]	1.3
2	Culture filtrate only	3.5
	0.05 Per cent pepsin only	0.0
	Culture filtrate + pepsin	1.0
3	Culture filtrate only	3.5
	0.05 Per cent trypsin only	0.0
	Culture filtrate + trypsin	2.5
4	Culture filtrate only	2.5
	0.5 Per cent erepsin only	0.0
	Culture filtrate + erepsin	0.0

[a] Cysteine-activated papain was used.

[b] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2 = browning in the lower ⅓ of the stem; 3 = browning in the lower ⅔ of the stem; and 4 = browning from the base to the apex of the stem. Figures are the averages of 4–5 plants.

TABLE 2.—*Browning and wilting activity of commercial enzyme preparations*

Name of enzyme preparation	Concentration (per cent w/v)	Wilt index[c]	Browning index[d]
Diastase[a]	0.5	0.3	0.0
α-amylase[a]	0.25	0.3	0.0
β-amylase[a]	0.25	1.0	0.0
Lipase[a]	0.25	2.0	0.0
Pectinol 100 D[b]	0.25	3.0	3.3

[a] Nutritional Biochemical Corporation.

[b] Rohm and Haas.

[c] The severity of wilt was determined on an arbitrary basis of 0 to 4, 0 indicating no wilt and 4 indicating maximum wilt. Figures are the average of 4–5 plants.

[d] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or in some leaf axils; 2 = browning in the lower ⅓ of the stem; 3 = browning in the lower ⅔ of the stem; and 4 = browning from the base to the apex of the stem. Figures are the averages of 4–5 plants.

when heated failed to produce either wilting or browning of tomato plants.

In working with *Pythium debaryanum* Hesse, Gupta (16) has observed that this organism produces an enzyme which reduces the viscosity of a pectin solution without releasing appreciable amounts of reducing groups. He was unable to show the presence of monogalacturonic acid liberated as a result of enzyme action. On the basis of this evidence he postulated that *P. debaryanum* produces no polygalacturonase but rather a depolymerase enzyme similar to the one reported by Beaven and Brown (1) in *Byssochlamys fulva* Olliver & Smith and by Wood (38) in *Erwinia aroideae* (Town.) Holland. As the enzyme obtained from the tomato wilt fungus produced only small amounts of reducing groups it was considered that this enzyme also might be a depolymerase. This preparation when incubated with a sodium polypectate solution reduced the viscosity of the solution, an indication that the polymer was being broken down. However, monogalacturonic acid was not the ultimate product of hydrolysis, as shown by the following experiment. Pectin and the enzyme preparation were incubated for 5 days at 30°C under sterile conditions. Aliquots of the mixture were withdrawn asceptically at intervals of 0, 1, 2, and 5 days. Each was heated to inactivate the enzyme, and then tested for monogalacturonic acid by means of paper chromatography. No monogalacturonic acid was detected. Similar experiments run with a commercial enzyme preparation showed a positive test for monogalacturonic acid at the end of 5 hours. It is evident that the pectin-splitting enzyme of *Fusarium oxysporum* f. *lycopersici* is a depolymerase (referred to herein as DP) rather than a polygalacturonase which breaks the pectic acid molecule into monogalacturonic acid.

It was of interest to determine whether or not PME free of PG and DP activity could produce both wilt and vascular discoloration typical of diseased plants. The presence of pectin polygalacturonase in higher plants has never been satisfactorily demonstrated except in germinating barley (24, 25). PME free from PG and DP was prepared from tobacco leaves according to the method described by Gupta (16). This enzyme preparation, with a PME activity of 2276 (determined at pH 7.0), was tested at 0.1, 0.05, and 0.01 per cent concentrations on tomato cuttings. No wilt was observed at any of these levels.

As reported previously (15), it is possible to produce vascular browning in healthy tomato stems by introduction of phenolic compounds into the transpiration stream. A wide variety of phenolic compounds are capable of being oxidized by the plant, thus producing the characteristic browning observed in the diseased plant (Table 3). Catechol and hydroquinone at higher concentrations produce both wilt and browning, but at lower concentrations they produce only vascular browning. Similar results were obtained by Davis, Waggoner, and Dimond (3).

TABLE 3.—*Production of wilt and vascular discoloration in tomato cuttings by different phenolic compounds*

Name of compound	Concentration (per cent w/v)	Wilt index[a]	Browning index[b]
Hydroquinone	0.05	4.0	4.0
	0.01	3.6	2.6
	0.005	0.0	1.0
Catechol	0.01	3.3	4.0
	0.005	0.0	2.3
	0.001	0.0	1.0
Phloroglucinol	0.05	3.0	2.6
	0.01	2.0	1.0
Phenol	0.05	4.0	2.0
	0.01	1.6	1.3
Resorcinol	0.05	4.0	4.0
	0.01	1.3	2.0
	0.005	0.0	1.0
p-Cresol	0.01	2.0	2.0

[a] The severity of wilt was determined on an arbitrary basis of 0 to 4, 0 indicating no wilt and 4 indicating maximum wilt. Figures are the averages of 4–5 plants.

[b] The intensity of vascular browning was graded as follows: 0 = no vascular browning; 1 = slight browning at the base of the stem or on some leaf axils; 2 = browning in the lower ⅓ of the stem; 3 = browning in the lower ⅔ of the stem; and 4 = browning from the base to the apex of the stem. Figures are the averages of 4–5 plants.

The results presented so far show that fungus enzyme preparations showing PME and DP or PG activity are capable of inducing vascular browning and wilt. PME that was free of PG or DP induced no wilting. Several phenols introduced into the transpiration stream caused vascular browning. The data obtained with enzyme preparations and phenols suggest that browning is the result of the action of polyphenol oxidase, known to be present in tomato stems (22), on phenols which were liberated into the vascular system by the action of pectic enzymes on the xylem walls. Davis et al (3) detected β-glycosidase in the vascular sap of diseased plants but not in that of healthy plants. They concluded that fungus β-glycosidase hydrolyzes β-glycosides of the host to phenols, that these phenols are oxidized by the polyphenoloxidase system of the host to melanin pigments, and that these pigments produce the characteristic vascular browning of the xylem.

The possible importance of β-glycosides in this relation was investigated. The method of Bourquellot (13) was used for the detection of the β-glucosides. No β-glycosides could be detected in homogenates of healthy tomato stems 6–8 weeks old. Tomato cuttings treated with 0.25 per cent solution of emulsin (400 units per g) showed no vascular browning. Although all the commercial pectic enzyme preparations tested showed varying amounts of β-glycosidase activity, there was no correlation between β-glycosidase activity *in vitro* and severity of disease symptoms. These results suggest that β-glycosidase is not directly concerned with the induction of disease symptoms.

DISCUSSION. — Pathologists have debated for a number of years whether or not a portion of the

syndrome of certain plant diseases may be caused by toxic substances produced in the host by the pathogen. Such substances have been called toxins. In some diseases, toxins are thought to cause a more rapid and extensive invasion by the pathogen than would be the case in the absence of a toxin. In other diseases, toxins are thought to be translocated from the center of infection and to produce conspicuous disease symptoms which are thus of secondary origin, but of primary consequence to the infected host.

When polysaccharides enter the vascular elements of tomato cuttings they cause wilting (17). Polysaccharides have been isolated from cultures of bacterial plant pathogens and from wilt-producing fungi (32). In vascular wilt diseases where the pathogen grows in vessels, it seems possible that polysaccharides can be liberated into the vascular system in sufficient amounts to cause wilting. The effect of polysaccharides in conductive tissues is probably physical. The polysaccharide molecules could aggregate and block the xylem elements producing water shortage. Other polymers produce similar effects on cuttings when their molecular dimensions approach those of polysaccharides. Although we do believe that wilting in tomato plants is the result of plugging of the vascular elements by aggregation of large molecules, we do not think that these large molecules are synthesized by the pathogen since the presence of polysaccharides cannot be shown in culture filtrates of *Fusarium oxysporum* f. *lycopersici* until the end of the third week (4). Replacement culture filtrates do not contain any appreciable quantities of polysaccharides. Furthermore the nontoxic effect of heated culture filtrates could hardly be explained on the basis of polysaccharides being chiefly responsible for wilt.

In the Fusarium wilt of tomato there is a high correlation between vascular discoloration and occlusion of vessel elements. Wilting in oaks infected by *Endoconidiophora fagacearum* Bretz has been shown by Struckmeyer and coworkers (31) to be due to plugging of the xylem vessels with tyloses and gums. Impeded movement of solutes in the transpiration stream has also been demonstrated by use of radioisotopes in studies on oak wilt (1) and on Fusarium wilt of tomato (6). The mechanism by which the translocative dysfunction and the ensuing vascular discoloration is brought about is of interest because it is operative in a number of diseases. Ludwig (23) found that before Fusarium-infected vessels discolor, they contain a viscous, transparent occluding substance which he thought was removed from microscopic sections during fixation and staining. Because the occluding material frequently fills a vessel even though the mycelium can rarely be found as an occluding agent, it was concluded that a vivotoxin must be operative.

Such a vivotoxin could well be a hydrolytic enzyme produced by the fungus and capable of attacking some component of the wall of the vessel. The experimental evidence indicates that the enzymes in question belong to the pectic group of enzymes. These pectic enzymes satisfy the essential criteria for being classified as vivotoxins: 1) Their presence can be demonstrated in culture filtrates, 2) they can be isolated from culture filtrates and their toxic effects on plants demonstrated, and 3) The presence of fungal pectic enzymes has been shown in diseased plants but not in healthy plants (36).

It appears that the cell wall is the principal site of attack. Pectic substances occur in most, perhaps all, plant tissues. Generally speaking, they are found in relatively large amounts in succulent soft tissues composed chiefly of primary walls, and under conditions of rapid growth and high water content. In older tissues wherein the cell wall is composed of 2–3 layers, the pectic substances are found in the middle lamella and in the primary cell wall, whereas the secondary cell wall contains practically no pectic substances. In this connection it would be relevant to mention that in the greenhouse this disease can be induced readily in young tomato plants (4–6 weeks old), whereas old plants (8–10 weeks old) inoculated under similar conditions come down with the disease slowly and sometimes not at all.

A breakdown of the pectic material of the cell wall could bring about wilting in 2 ways: 1) large fragments broken off the cell wall could block the xylem elements thus resulting in water shortage in the upper portion of the plant and thereby producing wilt, or 2) a breakdown of pectic materials may result in a disorganization of the water translocating system thereby producing wilt. Evidence obtained points to the first explanation as the more feasible.

Wilting in diseased tomato plants thus appears to result from a plugging of the vessels by degradation products of the cell wall which are liberated by pectic enzymes secreted by the fungus and acting on the constituents of the cell wall. The fact that the active pectin-splitting enzyme is a depolymerase instead of a polygalacturonase is very significant. The breakdown of the pectin of the cell wall into small soluble fragments by a polygalacturonase might not cause plugging since these smaller fragments might be expected to move freely in the transpiration stream. On the other hand, the larger fragments produced by depolymerase would more readily increase the viscosity of the transpiration stream until mechanical plugging occurred. The observation that a pectin methyl esterase free of polygalacturonase or depolymerase cannot produce wilting is further indication that the pectin in the cell wall is broken down. Our initial observations of the importance of the pectic enzymes, particularly depolymerase, of *F. oxysporum* f. *lycopersici* in producing vessel plugging and wilting were subsequently extended by Winstead and Walker (37) to several other Fusaria. It thus appears that the formation of pectic enzymes may play an important role in producing the wilting symptoms of many other plant diseases. Microchemical studies of the action

of pectic enzymes on vascular cell walls of the tomato will be reported in a subsequent paper. Recently Gäumann and coworkers (12) have isolated from crude filtrates of *F. oxysporum* f. *lycopersici* and of 2 other organisms a substance, vasinfuscarin, which was capable of browning the xylem elements of the tomato plant. No suggestion was made as to the mode of action of vasinfuscarin, although it was suggested that it may be an enzymatic protein. Vasinfuscarin might well be a pectinase since the method used for its separation is very similar to the one used by us for separating the pectic enzyme preparation from filtrates of the tomato wilt fungus.

The significance of the correlation between vascular discoloration and pectin methyl esterase activity is not readily understood. Although vascular discoloration probably is caused by the plant polyphenol oxidase system, the source of the substrate for this enzyme system still remains undetermined. Our experimental evidence would seem to rule out β-glycosides as being the source of phenolic compounds. Davis and Dimond (2) have found that diseased plants contain less lignin than healthy plants and suggested that the decrease in lignin is due to the shunting of phenols to melanin formation at the expense of lignin synthesis. Until further experimental evidence is forthcoming, the source of phenolic compounds will remain uncertain.

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VERTICILLIUM WILT OF THE STRAWBERRY WITH SPECIAL REFERENCE TO RESISTANCE [1]

Stephen Wilhelm

SUMMARY

Verticillium wilt occurs generally throughout California on strawberry and is the most serious of the diseases of that crop caused by a soil-borne pathogen. A method of rapidly eliminating susceptible plants from a mixed population is described. As defined herein, susceptible plants become infected and manifest symptoms upon the first or second greenhouse inoculation, and tolerant plants upon the third. Plants rated as resistant manifest no readily evident symptoms throughout the 3 inoculations, although some may become infected. Resistant plants possessing a higher level of Verticillium tolerance than the parents from which they were derived constituted 0 per cent of the progenies of susceptible by susceptible parents, 8.3–20.8 per cent of the progenies of susceptible by resistant parents, and 21.0–53 per cent of the progenies of resistant by resistant parents. In general, plants rated as resistant to Verticillium wilt in the greenhouse tests showed no symptoms in infested field soil in the probable presence of other strains of Verticillium, and those rated as resistant pathogen-invaded in the greenhouse generally threw off the infection under similar field conditions.

Factors for Verticillium wilt resistance appear to be dominant (over those for susceptibility) and quantitative (as evidenced by the expression of degrees of tolerance or resistance), and there appear to be linked with them, to a strong degree, other fatcors responsible for resistance to powdery mildew of the foliage.

Verticillium wilt resistance occurs in a few strawberry varieties such as Marshall, Sierra, and Blakemore, and in certain North American clones of *Fragaria chiloensis*. The evidence suggests that from this last named source, resistance possibly was introduced into the cultivated strawberry and carried in some of the early parent families from which the Sierra variety originated. Seedlings of a South American form of *F. chiloensis* and of *F. virginiana*, ancestors of the garden strawberry, and seedlings of 4 relatively old English varieties were all susceptible.

Verticillium wilt of strawberry caused by *Verticillium albo-atrum* Rke. & Bert. was described in detail by Thomas in 1931 and 1932 (21, 22). This disease occurs generally throughout California, and was known among growers prior to 1931 as strawberry brown blight (11). Verticillium wilt is now recognized in New York (4), Oregon (12), Michigan (9), England (10, 13), Holland (15), and New Zealand (6). In California, it continues to be the most important disease of strawberry caused by a soil-borne pathogen. Because of pathogenicity for important agricultural crops and some weeds and the exceptional longevity of the microsclerotia (18), *V. albo-atrum* is wide-spread and persistent in many of California's soils. It is unaffected by the majority of common soil fumigants, except at comparatively high dosages (26), and rotations with nonsusceptible crops and/or fallow have not generally given the control reported elsewhere (17, 27).

Resistance to Verticillium wilt as possessed by certain clones of *Fragaria chiloensis* (L.) Duch., such as one from Oregon (23), and by the varieties Marshall and Blakemore was noted in the early work of Thomas (22, 24). Thomas also alluded to a quantitative type of inheritance by showing that resistance and susceptibility grade into each other with continuous variation, as is often characteristic of inheritance in polyploid plants. Crosses between *F. chiloensis* and susceptible varieties such as Nich Ohmer and Redheart made by Thomas and Goldsmith around 1930, or with types with *F. chiloensis* parentage developed by Etter (5), or with Marshall (24) could have incorporated Verticillium wilt resistance into certain of Thomas's early parent families. In 1945, Sierra, a highly resistant variety, was released by the California Agricultural Experiment Station (24).

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Climatic conditions and host nutrition greatly influence symptom expression. A lush vegetative plant grown with high nitrogen is more affected than one grown hard. This observation is in agreement with results published by others on the Verticillium wilt disease of potato (8), of hop (14), and of tomato (19). Cool overcast weather in the spring, interspersed with warm bright days, favors symptom expression, especially if the infected plant is bearing fruit.

Although the pathogen is soil borne, Verticillium wilt of strawberry typically is irregularly distributed in the field, even on heavily infested lands. This tendency of plants to escape infection is one of the greatest difficulties in accurate testing of individuals for resistance or susceptibility in the field (2, 3). The method reported herein obviates this difficulty.

Inoculation technique and rating.—Roots of 2- to 3-month-old seedlings or 1-year nursery plants are washed and then dipped in a spore suspension of a virulent culture of the conidial component of *V. albo-atrum* initiated by single-spore transfer. Plants thus inoculated are planted in 4-in. pots. The soil mixture used throughout these tests was patterned after that devised by Lawrence and Newell, known as the John Innes Mix (16), in which a consistent and fairly high nitrogen level is maintained by decomposition of hoof and horn grist. Initial partial sterilization is obtained with chloropicrin. In Berkeley the first inoculation is made in March or April when the weather is cool and usually fluctuates from overcast to clear and warm.

Susceptible plants began to wilt within 6-8 weeks after the first inoculation (Fig. 1, A). At the time when such plants were discarded, a culture was made of a petiole of each, thus providing an accurate means of definitely linking symptom expression and infection with Verticillium. Culturing was done by placing cut pieces of petioles on plain agar to which barley straw sterilized with propylene oxide had been added (Fig. 1, D). Infected petioles often showed pronounced black streaks on upper and lower sides (Fig. 1, B).

Plants surviving the first inoculation or which gave an uncertain reaction were inoculated a second time, usually after 8 weeks. In this inoculation the exposed roots were sprayed with a spore suspension as the plants were shifted to 6-in. pots. Plants showing symptoms following either the first or second inoculation, and yielding the pathogen in culture, were classed as susceptible (Fig. 1, C).

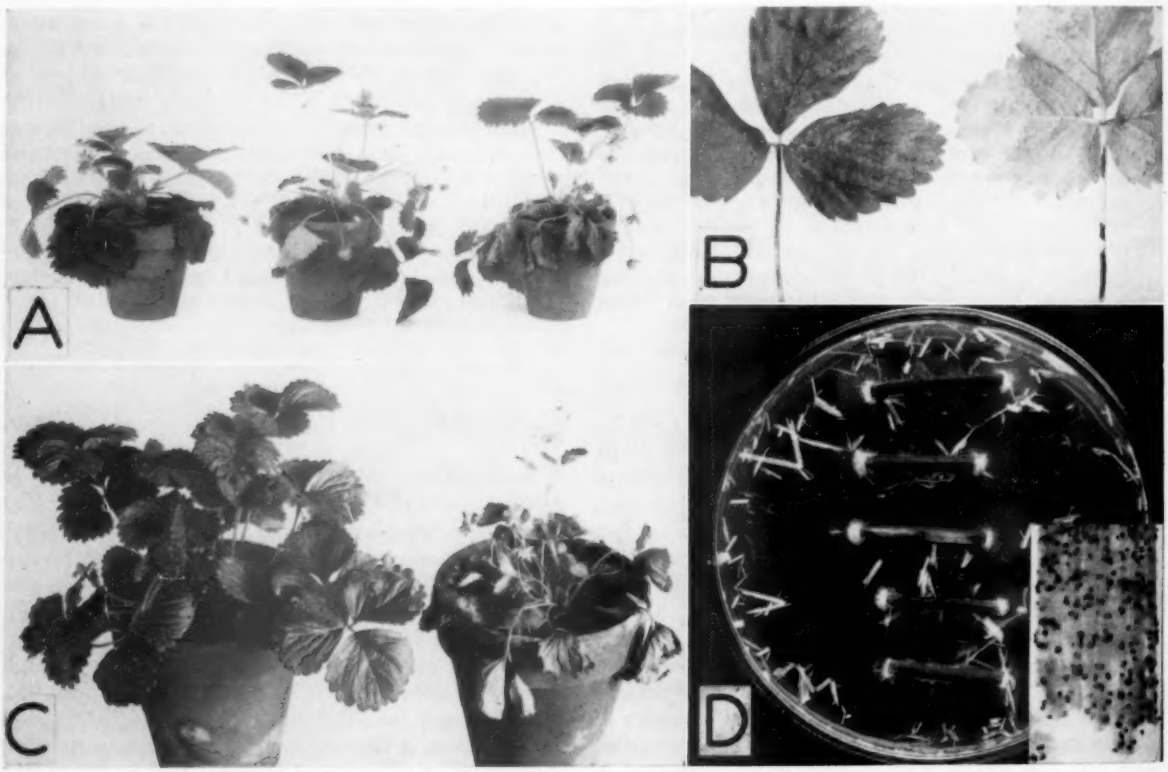

FIG. 1. A) The Verticillium susceptible reaction in 3 strawberry seedlings 6 weeks after the first inoculation. Note the collapse of lower leaves. B) Leaves of susceptible seedlings showing black streaks on the upper (left) and lower (right) surfaces of the petioles. C) Right, the susceptible reaction 8 weeks after the second inoculation. Left, a resistant plant. D) Petri-dish culture showing reisolation of Verticillium on propylene-oxide-sterilized fragments of barley straw in 2 per cent agar from 5 infected petioles of susceptible plants. Lower right, microsclerotia of Verticillium (enlarged) as they form on a fragment of barley straw in the agar, facilitating rapid detection of the fungus.

Plants surviving the second inoculation without symptoms were similarly inoculated a third time and shifted into 8-in. pots. Those developing symptoms, which at this stage were usually mild, were classed as tolerant and discarded.

Plants withstanding all 3 inoculations without manifesting symptoms were classed as resistant. At the end of the tests, 5 petioles of each such plant were cultured to determine whether or not invasion had occurred. The following year these plants, both invaded and pathogen-free groups, were bare-rooted and the crowns divided. One crown division of each was planted in the field in Verticillium-infested soil, and a sister crown was reserved for further greenhouse testing.

Inheritance of resistance.—Progenies of 13 crosses, and several other selfed populations along with asexual clones of some commercial varieties of strawberry and *Fragaria* species were studied. The ratings of the parents (Table 1) as to susceptibility and resistance had been determined previously by Harold E. Thomas, Director of the Strawberry Institute of California, and were based upon several years of field testing. When inoculated as described above, those rated as resistant readily became infected and manifested varying degrees of symptoms, but tolerated the infection without serious stunting. In the classification used herein they would be considered tolerant.

Progenies of crosses 1–4 involving susceptible parents, selfed British varieties, or selfed Lassen (Table 1) gave a predominance of susceptible individuals. No plants were resistant. Three per cent of the progeny of cross 1 fell into the tolerant class. It should be pointed out that 2 of the grandparents, 1 on the maternal and 1 on the paternal side, possessed some tolerance.

Among the progenies of crosses of susceptible and resistant parents (Table 1, crosses 5–8), greater than 50 per cent were susceptible, and the remainder were distributed about equally in the tolerant and resistant classes.

Among the progenies of crosses of resistant parents (Table 1, crosses 9–13), fewer than 50 per cent were susceptible, and the remainder were distributed about equally in the tolerant and resistant classes.

All of the 39 seedlings of *F. virginiana*, the 6 plants of 2 clones of *F. virginiana*, and the 12 plants of a clone of *F. vesca* were susceptible. Twelve plants of *F. chiloensis* collected in the wild near Bodega Bay, California, were resistant, whereas 43 seedlings of a South American form grown at Ambato, Ecuador, were all susceptible. An asexual clone of a variety developed by Etter, possibly Ettersburg 121, was susceptible. This finding was in agreement with earlier observations of H. C. Smith of New Zealand (20) from whom the plants were obtained. The Blakemore and Sierra varieties were rated as resistant though they were occasionally invaded by Verticillium. Blakemore's parents, Missionary and Howard 17, were susceptible.

Cultures showed that 25.4 per cent of the 397 plants rated as resistant (Table 1) acutally were invaded by the Verticillium fungus. Invasion may have occurred through open ends of vessels broken during the repotting and inoculation procedures, and may not reflect the capacity of the fungus to penetrate uninjured roots.

Field behavior of resistant plants.—At the end of 1 year's growth in Verticillium-infested soil in the field, 78 per cent of the 101 resistant pathogen-invaded plants had become pathogen-free as revealed by the culturing of 5 of the outer petioles of each. The remaining 22 per cent retained the pathogen, and most of these manifested at some time faint to moderate symptoms. This indicates that these latter should be reclassed as tolerant.

The great majority, 87 per cent, of the 296 plants rated as resistant pathogen-free remained so during the field test. Only 13 per cent became infected, and only half of these showed faint symptoms, notwithstanding the fact that strains of Verticillium with pathogenic capabilities different from those of the one used in the greenhouse testing undoubtedly were present in this field.

General characteristics of resistant plants.—Resistant plants, regardless of the parentage, generally possessed dark green glossy foliage suggestive of *F. chiloensis* as found in parts of coastal California. Leaflet characteristics ranged from dark bluish green and a tendency to cup downward, to medium deep green and a tendency to cup upward. The leaves generally were noticeably resistant to powdery mildew, both under field conditions and the more favorable conditions prevailing in the greenhouse. Approximately 5 per cent of the resistant plants were susceptible to mildew, and many of these occurred in the cross in which Lassen (very susceptible to mildew) was used as a parent.

Discussion.—In view of the generally variable results obtained from field tests for Verticillium wilt resistance in strawberry, the greenhouse method has many advantages. Foremost are the rapidity with which susceptible individuals can be eliminated from a segregating population and the possibility of quick recognition of a high degree of resistance. In the practical application of this method, culturing could be largely omitted, although it is important to know which individuals classed as resistant may have been invaded. A drawback may be the extreme severity of the test. Desirable plants with considerable field resistance or with a strong tendency to escape the disease may possibly be eliminated.

The susceptible and tolerant classes contained degrees of disease reaction varying from little or no tolerance to high tolerance as evidenced by symptom expression. The resistant plants, even if pathogen-invaded, manifested no evident symptoms in the greenhouse, and tended to rid themselves of the pathogen in the field. A small percentage of the resistant plants

TABLE 1.—*Inheritance of Verticillium wilt resistance in the strawberry*

Cross No.	Cross or variety tested	No. of plants tested	Percentage of plants in indicated disease class			Percentage of resistant plants pathogen-invaded
			Susceptible	Tolerant	Resistant	
1	B18.19 (S) × B48.8 (S)[a]	101	97.0	3.0		
2	B22.15 (S) × B48.8 (S)	11	100.0			
3	J6 (S) × B28.4 (S)	60	100.0			
4	B48.8 (S) × Lassen (S)	147	100.0			
	Lassen (S) self	258	100.0			
5	B18.29 (S) × B75.6 (R)	77	59.7	19.5	20.8	56.3
6	B22.15 (S) × 794.19 (R)	72	85.1	3.9	11.0	
7	B75.6 (R) × Lassen (S)	96	83.1	8.4	8.3	25.0
8	J6 (S) × B57.122 (R)	616	81.6	9.6	8.8	6.5
9	B57.122 (R) × 794.19 (R)	74	38.0	29.5	32.5	33.3
10	B75.6 (R) × B57.122 (R)	102	25.5	21.5	53.0	9.3
11	B75.6 (R) × B90.11 (R)	81	40.7	38.3	21.0	23.5
12	B75.33 (R) × 794.19 (R)	595	37.8	24.2	38.0	30.6
13	B90.11 (R) × B57.28 (R)	89	48.4	25.8	25.8	21.7
	Royal Sovereign (S) self[b]	72	100.0			
	Sir Joseph Paxton (S) self	38	100.0			
	King of the Earlies (S) self	94	100.0			
	Waterloo (S) self	58	100.0			
	Fragaria virginiana (asexual)	6	100 0			
	Fragaria virginiana (seedlings)	39	100.0			
	Fragaria vesca seedlings	12	100.0			
	Fragaria chiloensis (asexual) (Bodega Bay)	12			100.0	
	Fragaria chiloensis (seedlings) (Ambato)[c]	43	100.0			
	Sierra (asexual)	12			100.0	16.6
	Blakemore (asexual)	12			100.0	25.0
	Howard 17 (asexual)	12	100.0			
	Missionary (asexual)	12	100.0			
	Etter's variety (121?) (asexual)	4	100.0			

[a] Numbering system as used by the Strawberry Institute of California, (S) means susceptible to Verticillium wilt, (R) resistant. Crosses were made by the late Mr. Earl V. Goldsmith.

[b] Seed of Royal Sovereign, Sir Joseph Paxton, King of the Earlies, and Waterloo were kindly supplied by Norman H. Grubb, East Malling Research Station, Kent, England.

[c] Seed supplied to the Strawberry Institute by George M. Darrow.

retained the pathogen in the field but did not manifest symptoms.

Plants classed as resistant in this paper were reported as immune earlier (25). They appear to possess a higher level of resistance, perhaps more factors for it, than their parents. This transgressive segregation, i.e. the phenotypic expression of a character in a hybrid having a value beyond the parental limits, is known in fruit characters of strawberry (1), but perhaps this is the first time it has been noted with respect to disease resistance in strawberry.

The occurence of susceptible plants among the progenies of resistant parents (Table 1, crosses 9–13) suggests that in inheritance Verticillium resistance in strawberry is dominant over susceptibility, and the continuous gradation from susceptible to resistant suggests a quantitative type of inheritance such as is known in some other octoploid plants of interspecific hybrid origin. Resistance also appears to be linked somewhat strongly with characters responsible for deep green glossy foliage and powdery mildew resistance, suggestive of derivation from *Fragaria chiloensis* as it occurs in areas along the California coast. Seedlings of 1 form of *F. chiloensis* from South America, with dull pubescent foliage (mildew susceptible), were susceptible to Verticillium wilt, as were 2 clones and several seedlings of *F. virginiana*. Also all of the British varieties tested by Harris (11) and by Keyworth and Bennett (13), and the selfed seedlings tested in this study proved susceptible. Some of these were originated within only a relatively few years of the renowned Keen's seedling (1821) considered to be a principal progenitor of the present large-fruited types of strawberry. These facts together with the known history of the development of the strawberry in California suggest that factors for Verticillium resistance might have been introduced into the garden strawberry on several occasions and that resistance possibly came from North American forms of *F. chiloensis*.

The fact also herein developed that susceptible parents can yield progeny with a higher degree of resistance than either of them possesses, particularly as in the case of Blakemore, suggests that they may carry factors for resistance. These observations are well within the theoretical possibilities of an octoploid plant. For instance, assuming that the factors for Verticillium wilt resistance are located in 1 genome, a simplex or duplex condition homozygous for the resistance factor may give a susceptible disease reaction, but in crosses with genetically similar material, individuals quadriplex for the resistance factor may

result. Quadriplex individuals may give the tolerant or resistant reaction, or at least would likely be more resistant than the simpler parents. Also, inasmuch as allosyndesis is assumed to occur rather commonly in strawberry, it may be posible to obtain even a higher than quadriplex condition with respect to the resistance factors.

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APPLE BLACK ROT IN GEORGIA AND ITS CONTROL [1]

Jack Taylor [2]

SUMMARY

This investigation has brought out additional disease symptoms and phases of the apple black rot cycle not previously reported. Platings of fruit parts over a period of 3 years show that black rot infection may occur throughout the season from the time the buds break in spring until harvest.

Early symptoms appear as small purple spots on leaves and sepals. These later enlarge and develop tan centers. Secondary growth causes a frogeye spot on leaves and a blossom-end rot on fruit. Early fruit infections appear as small purple pimples which crack slightly and cause rotting later when the fruit begins to ripen. Late-season infections appear as irregular black spots which enlarge slowly while the fruit is green and increase progressively as it ripens. The core-rot phase of the disease is characterized by premature coloring and dropping of fruit. These symptoms appear 3–6 weeks before harvest and may cause considerable loss on the Red Delicious variety of apples. When the necessary sanitary measures were used, captan gave economic control of the fruit rot and leafspot. It was applied at 6- to 8-day intervals from the beginning of the prepink stage and continuing through the petal fall stage, and then at 2-week intervals.

Inoculation studies show that black rot is a ripe-rot type of disease. April infections will not cause severe rotting of fruit until 4–8 weeks before harvest, but inoculated ripe fruit will rot completely in 3–5 days.

Physalospora obtusa (Schw.) Cke., the cause of black rot and leafspot, caused greater loss to Georgia apple growers than did all other pests combined. An orchard survey in 1951 (17) showed that in some cases as much as 60 per cent of the fruit was lost to the fruit rot alone. According to Wolf (21) fruit losses of 25–50 per cent occurred in the South as early as 1912. During 1952 some growers lost 90–95 per cent of some varieties because of this disease (18). The extent of these losses is not fully realized by the grower because the fruit usually drops from the trees before or soon after the symptoms appear and quickly rots in the moist environment of the ground cover.

Others have found that fungicides are of little value in controlling the fruit rot because the organism enters the fruit through unprotected injuries (3, 7, 11). Some spray bulletins do not suggest black rot as a problem until the sixth cover spray (1).

The present study was initiated during 1952 in an attempt to discover additional etiological facts that would serve as a basis for better control methods. This paper presents evidence to show that infections with the apple black rot organism may occur throughout the growing season in Georgia although rotting symptoms do not appear until the fruit begins to ripen, and that the disease may be controlled economically by proper orchard sanitation and timely applications of effective fungicides.

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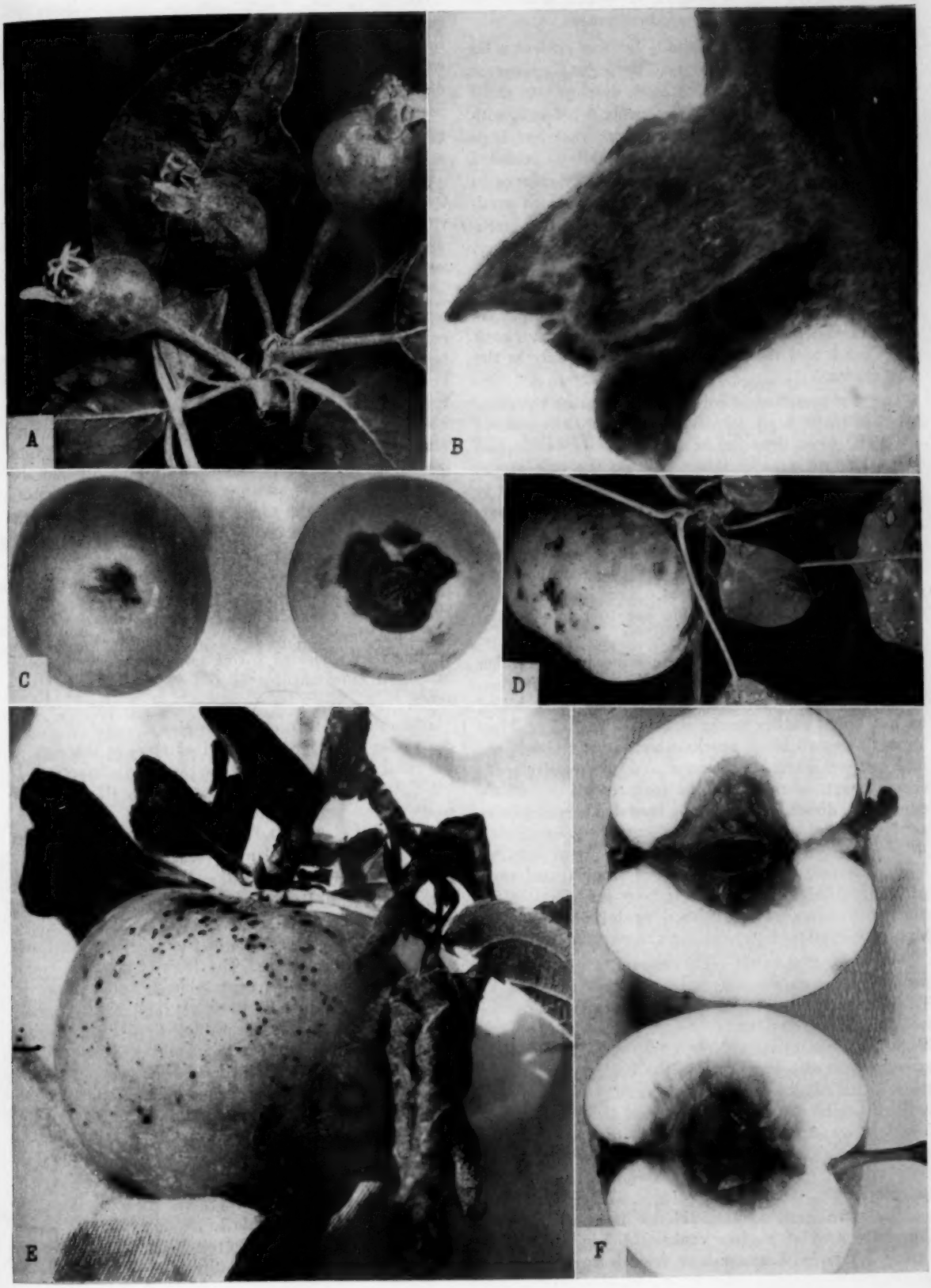
SYMPTOMS.—Leaf symptoms usually appear early in the spring shortly after the leaves emerge. They are at first minute purple spots which enlarge rapidly and reach diameters averaging 4–5 mm. Later these spots become tan and more definitely delimited from the remainder of the leaf. After remaining in this condition for a few weeks, many of the spots begin a secondary enlargement in an irregular manner, forming lobes or more or less concentric rings. The first 2 stages of leafspot symptoms are shown in Figure 1, A and later stages of development in Figure 1, D.

On sepals, the symptoms first appear as minute red specks that later turn purple and usually are bounded by a red ring (Fig. 1, B). Still later the center of the spots changes to a yellowish brown with a purple external ring usually bordered by a slight red circle. After a few weeks the entire sepal lobe becomes affected and becomes dark brown. Fruit enlargement is often restricted at the infected sepal. This causes a lopsided apple. Fruit-rotting symptoms begin to appear early in June on varieties ripening in that month, late in June on varieties ripening in the middle of August, and early in July on varieties ripening in late August and September. Sepal infections cause a

D——→

FIG. 1. Early- and late-season apple black rot symptoms on fruit and frogeye on leaves. A) Detroit Red showing pimples on fruit and first 2 stages of frogeye on leaves. B) Sepal infection on Detroit Red at 1 week after petal fall. C) Blossom-end rot (June 6) from sepal infections as shown in B. D) Red June showing rot development on fruit and frogeye on leaves from early-season infections (note growth restrictions at areas showing numerous infections). E) Late-season infections on Stayman Winesap by inoculum produced on twig (shown) killed by fire blight earlier in season. F) Core rot in Red Delicious.


A
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blossom-end type of rot (Fig. 1, C) which usually develops before infections from other centers.

Symptoms on the fruit usually become evident a little later than leaf symptoms. The lesions appear as minute reddish specks which later develop into small purple pimples (Fig. 1, A) measuring 0.1–1 mm, with slightly sunken tan centers. On fruit that has been exposed to direct light, the pimple usually is bounded by a narrow red ring. It does not usually enlarge in diameter for several weeks but many increase vertically and rupture the epidermis transversely or circularly. This may appear as a wound or as an injury. Numerous infections appear as rough russeted areas without any sign of rotting until the fruit begins to ripen or until after harvest. Growth at affected areas may be restricted (Fig. 1, D), and in many cases such areas crack and the rot then advances rapidly as the fruit ripens.

The first symptom of core rot is premature ripening. Infected fruits begin to color 3–6 weeks before sound ones, and often drop before the rot shows on the surface. The rot usually shows first on the side of the fruit, while the blossom and stem ends are the last to be invaded. After the fruit drops, it rots in a few days. Core rot may start at any point in the central core chamber (Fig. 1, F) or along the sinus connecting the core with the calyx cup. It usually begins about the same time as blossom-end rot. The Red Delicious variety is the most susceptible one to this phase of the disease.

Some varieties such as Stayman Winesap and Golden Delicious are very susceptible to late-season infection. These infections are most common near twigs killed by fire blight earlier in the season (Fig. 1, E). They appear as small black specks which at first slowly enlarge in an irregular manner and progressively increase in rate of rotting as the fruit ripens. Infections exposed to direct light often are bounded by red whereas this coloration is absent in infected areas on shaded sides of the same fruit. Symptoms usually appear the latter part of July and first part of August, and such fruit are worthless by the time they are ripe. Infections also occur at any wound or injury and show black rot symptoms typical for the particular variety.

Source of inoculum.—The amount of inoculum depends largely on the amount of available dead bark. The organism seems to grow more profusely in quick-killed bark such as that found on twigs killed by fire blight or by pruning, but cankers caused by *Physalospora obtusa* also are found in the bark of most older trees. The organism sporulates most abundantly in bark of fire blight cankers. Blighted twigs measuring 5 mm in length and about 3 mm in diameter showed an average of 366 pycnidia, each containing about 1200 conidia. The count areas were selected from twigs where pycnidia were evident. These pycnidia usually were grouped and did not necessarily cover the entire area of a given canker. A 1-in. twig measuring 3 mm in diameter may furnish approximately 2,000,000 spores. Measurements of blighted limbs and twigs from a Stayman Winesap tree taken at random in a Fannin County orchard showed 2440 in. of dead wood measuring 2–15 mm in diameter. This amount of dead wood may yield 4,880,000,000 spores during 1 season. Fruit directly under twigs which apparently produced 10,000,000 spores showed a range of 0–56 infections, with most fruit showing 3–4 infections. This possibly explains unsatisfactory results of surface-inoculation attempts. The usual application of spores would range from 200–500 per fruit in comparison with 1–4 million available to fruit under dead twigs measuring 1–2 in.

The amount of available black rot inoculum is greatest in early spring. Twigs killed by fire blight and collected in January contained mature conidia which began to germinate within 30 minutes with over 90 per cent germinating in 24 hours. No change was found in February collections, but material collected March 27 showed 50–80 per cent empty pycnidia with ascocarps beginning to develop. Observations on April 26 showed about 90 per cent empty pycnidia and mature perithecia and ascospores. The date of the spring discharge of conidia varies with the season. According to Hesler (11) the ostiole does not open at temperatures below 60°F or in the absence of moisture, and a period of humidity of several hours is necessary. In Georgia, twigs killed by fire blight in early spring showed mature conidia in the latter part of the following June during 1952 (18), 1953, and 1954. These conidia were liberated during rainy periods and caused infections on young leaves and unprotected fruit surfaces.

Isolations.—During 1952 apples were taken at random from unsprayed Detroit Red trees at the time of the petal fall and first cover sprays and plated on water agar. The fruits were plated with all the flower parts except petals. About 18 per cent of these platings showed *P. obtusa.* An *Alternaria* sp. was almost invariably present, and many other fungi were found.

The 1953 platings were made of fruit from unsprayed Detroit Red trees when buds were pink, at petal fall, and 30 and 60 days after petal fall. The 2 later platings were sections of fruit showing small, almost microscopic specks. The fruit was selected from areas near dead twigs containing fruiting bodies of the black rot fungus. Of these platings, 7.6, 11.0, 77.2, and 69.2 per cent, respectively, showed *P. obtusa.* Petal-fall platings that contained withered flower parts almost invariably contained an *Alternaria* sp., but later platings showed organisms other than *P. obtusa* in less than 2 per cent of the plates.

The platings made in 1954 were similar to those of the previous year until symptoms became evident; then platings of sepals, core, and infected fruit were made. Platings were made of several varieties from various orchards as shown in Table 1. Sections of June apples plated June 16 and 25; Detroit Reds plated June 25, July 6, and August 2; and Stayman Winesap plated

TABLE 1. *Relative prevalence of Physalospora obtusa in early-season platings from several varieties of apples*

Date plated	County source	Variety	Parts plated	Percentage of plates showing		
				P. obtusa	Alternaria	Other organisms
4/26	Union	Detroit Red	sepals	10.0[a]	40.0	—
4/26	Union	Detroit Red	ovulary tissues	10.0	15.0	—
4/28	Union	Detroit Red	sepals	8.3	75.0	—
5/5	Union	Detroit Red	sepals	64.0	18.2	—
5/5	Union	Detroit Red	core, without pimples	15.0	—	—
5/5	Union	Detroit Red	calyx cup without sepals	33.3	16.6	23.6
5/5	Union	Detroit Red	fruit sections showing pimples	85.0	—	—
5/14	Fannin	Lodi	fruit sections showing pimples	85.0	—	—
5/14	Fannin	Lodi	sepals showing spots	77.7	77.7	88.9
5/25	Union	Paducah	sections showing pimples	68.7	—	—
5/25	Gilmer	Yates	sections showing pimples	100.00	—	—
5/25	Gilmer	Yates	tissue with pimple skin removed	—	—	—
5/25	Gilmer	Red Delicious	sections showing pimples	41.7	—	—
6/16	Fannin	June apples	sections showing pimples and enlarging specks	75.0	3.6	7.2
6/25	Fannin	June apples	blossom-end rot	95.0	—	5.0
6/25	Fannin	June apples	rotting from pimples	68.4	—	31.6
6/25	Fannin	Detroit Red	sections showing pimples	80.0	—	15.0
6/25	Fannin	Detroit Red	blossom-end rot	80.0	80.0	—
7/6	Fannin	Detroit Red	pimples, some showing secondary enlargement	87.9	—	12.1
8/2	Fannin	Detroit Red	pimples, some showing secondary enlargement	81.8	9.1	36.3
8/2	Fannin	Stayman Winesap	pimples, some showing secondary enlargement	96.0	4.0	4.0

[a] 100 platings were made on each date.

June 25 and August 2 were from fruit showing secondary enlargements from pimples as shown in Figure 1, D or blossom-end rots from infected sepals.

INOCULATIONS.—Wound inoculations of green and ripe Red Delicious apples were made from cultures of *P. obtusa* on May 24. The green fruits were collected from a tree on that date and the ripe ones were bought from a local store. Ten of each type were inoculated and 2 of each served as controls. All fruits were placed in a moist chamber at room temperature. After 5 days all inoculated ripe apples were almost completely rotted, while the inoculated green ones and controls showed no rotting at the end of 5 and 12 days (Fig. 2, A). After 21 days, inoculated green apples showed rotted areas measuring 3–12 mm in diameter and averaging 6.6 mm.

Red Delicious apples were inoculated in the field by placing mycelium from cultures of *P. obtusa* in wounds in the skin. Twenty fruits were inoculated on June 15, July 15, and July 31. Average lesion diameters 7 days after inoculation were 4.4, 16.4, and 34.2 mm, respectively. These fruits dropped from the tree and quickly rotted soon after the core was invaded by the organism. Twenty apples were wounded on each

FIG. 2. Fruit and culture inoculations. A) Green (upper) and ripe Red Delicious fruit (lower) 5 days after inoculation with *Physalospora obtusa*. B) Cultures of the fungus on media made from ripe (lower) and green fruit (upper).

date to serve as controls. Two of the controls showed rotting from wounds of July 15 and 3 from those of July 31.

An apple-agar medium made with 20 g agar and 200 g of apple to make 1000 ml of media was made from green and ripe Red Delicious apples. Ten plates of each were inoculated from a culture of *P. obtusa*. After 5 days, colony diameters for green- and ripe-fruit media averaged 54.0 and 78.7 mm, respectively (Fig. 2, B), with individual colony measurements ranging from 38.0–58.0 mm for green-fruit media and 74.0–84.5 mm for ripe fruit media.

CONTROL.—During 1952-54, fungicidal spray tests were made on Detroit Red apple trees. This variety is very susceptible to black rot and ripens during August in Georgia. The following fungicides at the stated dosages were used: 1) maneb or manganous ethylenebis [dithiocarbamate] (70 per cent), 1½–100; 2) ferbam or ferric dimethyldithiocarbamate (76 per cent), 1½–100; 3) captan or *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide (50 per cent), 2–100; 4) wettable sulphur paste (70 per cent sulphur), 6 or 8–100; 5) liquid lime sulphur, 1½–100; and 6) Bordeaux mixture, 2–4–100 in early sprays and 4–4–100 in later applications.

The fungicides for 1952 tests were selected from those used in toxicity studies of that year (19). Each of the folliowing treatments was applied to six 1-tree replicates in a randomized block: 1) maneb in 9 sprays, 2) ferbam in 9 sprays, 3) maneb plus sulphur in 3 sprays followed by maneb in 5 sprays, 4) ferbam plus sulphur in 3 sprays followed by ferbam in 5 sprays, and 5) sulphur in 3 sprays followed by Bordeaux mixture in 5 sprays. The sprays of treatments 1 and 2 were applied at about 1-week intervals for 4 sprays from the prepink stage of development through the petal fall period, and then continued at 2-week intervals for 5 cover sprays. In treatments 3, 4, and 5 the sprays were applied at about 2-week intervals throughout the season beginning at the prepink stage of development. The percentages of apples with black rot symptoms by harvest for treatments 1, 2, 3, 4, and 5 averaged 12.8, 26.7, 26.8, 26.1, and 34.6, respectively.

The 1953 spray tests were planned according to the 1-week schedule used in the 1952 test. In 1 test maneb and captan were applied in 4 sprays at 1-week intervals from prepink through petal fall and then continued at 2-week intervals for 5 sprays. With each of these treatments about 1 per cent of the fruits was affected with black rot while 24 per cent of the fruit on an unsprayed tree in the same block were affected. In another orchard where a grower had applied wettable sulphur paste in the prepink and pink sprays, the first test spray was applied to six 1-tree replicates at petal fall and continued at about 2-week intervals for 6 sprays. The treatments were as follows: 1) maneb, 2) captan, 3) ferbam plus sulphur in 2 sprays followed by ferbam alone in 4 sprays, and 4) sulphur in 2 sprays followed by Bordeaux mixture in 4 sprays. Fruit losses caused by black rot averaged 67.8, 69.5, 69.3, and 73.2 per cent respectively.

During 1954 captan was applied at 1-week intervals during prepink through petal fall and then continued at 2-week intervals. This schedule was compared with a treatment in which the same material was applied in the last 3 sprays. Fruit losses caused by black rot averaged 5.1 and 23.8 per cent, respectively. In another block of trees liquid lime-sulphur applied at 1-week intervals during the first 4 sprays followed by Bordeaux mixture in 5 sprays was compared with Bordeaux mixture in only the last 3 sprays. Fruits showing black rot symptoms by harvest averaged 7 and 12 per cent, respectively.

These data indicate that applications of effective fungicides at weekly intervals from the prepink through the petal fall periods will contribute to control of apple black rot. These results support the isolation data which show that much of the fruit infection of apples by the black rot fungus occurs early in the season.

DISCUSSION.—In other areas, *P. obtusa* apparently does not infect fruit before it begins to ripen and the fungus then enters through wounds and natural openings (3, 5, 6, 7, 11). Farther north the fruit rot apparently is not a problem. This may be because the spores are not liberated until after petal fall (14) when the fruit has become more resistant and when it has been adequately protected by fungicidal cover sprays.

There are wide variations in black rot symptoms among different varieties of apples, and also in symptoms within a variety, depending upon time and type of infection. Detroit Red and Red Delicious are among the varieties most susceptible to blossom-end rot. Stayman Winesap and Red Winesap are susceptible to infections on the body of the fruit, and Stayman Winesap and Golden Delicious are among the varieties most susceptible to late season infections. Red Delicious is the variety most susceptible to core rot. This apparently is because of the characteristic sinus connecting the central core chamber with the calyx cup.

Core rot is complicated by the presence of other organisms such as *Alternaria*, *Coniothyrium*, and *Fusarium sp.* Various bacteria and yeasts often cause the rotted tissues to have a slimy consistency. Carpenter (4) found that these organisms were associated with moldy core of apples in Wisconsin, but did not find that they would cause flesh rotting. Red Delicious apples often have moldy core in which the core cavities are overrun by a gray weft of mycelial growth. Flesh rotting occurs when *P. obtusa* is present. In some cases 20 per cent of Red Delicious fruit is lost to fruit rot.

Conidia appear to be the most important infective principle involved in apple black rot and frogeye leafspot. Inoculum is produced in abundance in early spring in prunings and in other dead wood and during

the summer in wood killed by fire blight the previous spring. Numerous reports have cited the relationship between fireblight and later development of frogeye leafspot. A recent report from Delaware (16) shows that this condition prevails in that area. Late-season fruit infections are not so troublesome in orchards that have escaped an epiphytotic of fire blight during that season. Removal of blighted wood soon after it is killed would contribute materially to control of late-season infections (Fig. 1, E).

Results of 1952-53 platings and of spray tests indicate that many of the infections by the black rot fungus occur before the first cover spray. This is the period during which elemental sulphur usually is used. Laboratory toxicity studies (19) indicated that elemental sulphur and lime-sulphur were not sufficiently toxic to conidia of the black rot fungus. Sulphur has not given satisfactory control of black rot and leafspot in experiments or in commercial orchards when applied according to the conventional schedule. One grower who used elemental sulphur in precover sprays followed by Bordeaux mixture lost at least 50 per cent of his crop in 1952. During 1953 this grower used 4 lb. of elemental sulphur plus ½ lb. of 50 per cent captan followed by 1 lb. of 50 per cent captan in later sprays and again lost about 50 per cent of his crop to the fruit rot.

The 1954 platings (Table 1) and observations agree with 1952 and 1953 findings and show that infections which cause black rot and leafspot occur during the same periods. In Georgia, infection may occur at any time after the buds break in the spring when temperature and moisture conditions are favorable. The tips of leaves and sepals are the first susceptible tissues exposed to infection. Under satisfactory growing conditions the buds open rapidly and new susceptible parts are exposed. When the blossoms open, all exposed flower parts are subject to infection. As the fruit and leaves become older they apparently become more resistant to infection except in cases where the skin of the fruit is injured either by spray materials or by other agents.

Inoculation studies show that the organism will not cause rapid rotting symptoms in green fruit but will almost completely rot ripe fruit within 5 days when placed in moist chambers at room temperature. Culpepper et al (8) found that there was a rapid decrease in the content of reducing sugars, disaccharides, and lipoid sugars as the black rot organism invaded the tissues, while the amount of starch remained unchanged throughout the process of decay. Krotkov and Helson (12) found that the sugar content of McIntosh apples began to increase rapidly as the fruit ripened, with the highest percentage by fresh weight being reached at or just after picking. The starch content decreased as the sugar content increased. Field observations show that rotting symptoms begin to appear 6–10 weeks before picking and progress slowly at first, with the rate of rotting increasing as the fruit ripens. After the fruit drops from the tree it rots completely in a short time. The inoculation studies agree with these observations in that the inoculated fruit rotted very slowly in May but the rate of rotting progressively increased as the fruit ripened. The ability of *P. obtusa* to rot fruit seems to be correlated with the sugar content of the fruit.

Results of field control tests are presented in support of studies on time of infection, but they indicate that economic control of apple black rot may be obtained by applying effective fungicides at weekly intervals during the period in which growth is most rapid. Sprays applied at weekly intervals from early prepink until petal-fall was complete have given a substantial decrease in black rot on Detroit Red apples. The removal of all dead wood and prunings from the orchard is the first step in controlling apple black rot, but in most older orchards it is economically impossible to reduce the source of inoculum sufficiently to obtain control with the conventional sulphur-Bordeaux mixture schedule. Timely applications of effective fungicides combined with the necessary sanitary measures may give economic control of apple black rot and frogeye leafspot in Georgia. There is a great need for a control of fire blight. This disease produces about 90 per cent of the substrate of *P. obtusa* in Georgia.

It has long been recognized that *Botryosphaeria ribis* (Tode ex Fr.) & Dug. produces a rot of apples that is almost indistinguishable from black rot (9, 20). The rot caused by *B. ribis* has not been of great importance in Georgia but may become a problem in the future. Others have recently reported *B. ribis* (10, 13) and *Botrytis cinerea* Pers. (15) as important causes of summer fruit rots of apples, but of 2100 platings made in Georgia during 1953-54, *P. obtusa* was found in over 80 per cent of the early-season platings. *B. cinerea* was found in less than 2 per cent of the sepal platings. *B. ribis* was found in 2 platings made during August, 1954. Other organisms have been found causing a blossom-end rot of apples in Georgia but they are not included in this report.

An *Alternaria* sp. was often found in plates containing withered flower parts and sometimes appeared in sepal platings, but was not found in fruit sections containing spots such as are shown in Figure 1, A.

GEORGIA MOUNTAIN EXPERIMENT STATION
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A LABORATORY METHOD FOR TESTING SOIL FUNGICIDES, WITH PHYTOPHTHORA CINNAMOMI AS TEST ORGANISM [1]

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SUMMARY

A laboratory method consisting of 2 phases is described for selecting effective soil fungicides. Materials were tested in glass vials containing 2 in. of autoclaved soil, with an inoculum disk placed at a depth of 1 in. Chemicals were applied 1) as drenches or 2) as dry mixes with the soil. Inoculum was recovered after a 24-hour incubation period, and placed on agar in Petri dishes to determine viability. The principal test fungus was *Phytophthora cinnamomi* Rands. *Verticillium albo-atrum* Reinke & Berth. was also used to a limited extent.

Many chemicals that are highly fungicidal on the basis of standard laboratory testing methods were ineffective when applied as drenches to soil. Only 10 of the 47 chemicals tested by the drench method penetrated 1 in. of soil in fungicidal concentration when applied to give a dosage of 500 ppm. The 2 most effective materials were sodium methyldithiocarbamate (Stauffer N-869 or Vapam) and tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione (Stauffer N-521), which were fungicidal at 5 ppm.

The 4 most effective fungicides in the dry-mix test were 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide (Acti-dione), ethylmercury phosphate (Ceresan, New Improved), Stauffer N-869, and Stauffer N-521, which killed *P. cinnamomi* at 5 ppm. Several chemicals that were ineffective in soil when applied as drenches were fungicidal in the dry-mix test, where penetration of soil is not a factor.

Materials that were fungicidal in the laboratory drench test also killed *P. cinnamomi* in soil in glasshouse tests. Materials selected as effective fungicides in screening tests in agar varied greatly in their performance in soil.

Striking differences were noted in the fungitoxicity of closely related compounds. Sodium methyldithiocarbamate was approximately 500 times more fungicidal than sodium dimethyldithiocarbamate or sodium diethyldithiocarbamate. Other examples are presented of similar relationships between structure and biological activity.

In the course of research on chemical control of root pathogens, it became evident that fungicide testing methods currently in use were inadequate for detecting effective soil fungicides. Fungicides that were highly effective against root pathogens on the basis of standard laboratory tests using the glass-slide, test-tube dilution, or Petri-dish methods gave little or no control of these same pathogens when applied to soil in glasshouse or field tests. This paper presents a laboratory method, consisting of 2 phases, that is designed to screen soil fungicides and to give a higher correlation between laboratory and field per-

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formance than do the standard laboratory testing methods. The method has been used primarily to select fungicides for application as drenches to the root zones of plants affected with root rots, but it also may be used to screen fungicides or fumigants for soil treatment in general. A preliminary note on this technique was published in 1952 (8).

Ezekiel, in 1938 (3), reported the laboratory evaluation of some soil fungicides and fumigants in soil. His work dealt with the effect of materials on a specific pathogen, and the methods were not suited to large-scale rapid screening tests. For these tests he used 1-qt. Mason jars. Chemicals either were mixed dry with the soil prior to addition of inoculum, or were applied as volatile materials to the soil surface after infestation of the soil. Arndt (1) has described an interesting method for evaluation of seed protectants against *Rhizoctonia solani*. He used aluminum dishes, planted cotton seed in a fungicide-sand mixture, and recorded invasion of the seedlings from inoculum placed near the center of the dishes. The method involved a 12-day incubation period and was not designed for screening of materials against a number of different pathogens. Klomparens and Vaughn (6) reported good correlation between laboratory performance of fungicides in malt agar and field control of several turf pathogens. With these pathogens, penetration of soil is not usually essential; hence a better correlation between standard laboratory tests and field performance might be expected than would be the case when dealing with root rot organisms.

MATERIALS AND METHODS.—The soil fungicide testing method described here involves the laboratory evaluation of fungicides in glass vials containing soil. The method is suitable 1) for testing penetration of materials applied to the soil surface in solution or suspension (as drenches), or 2) for testing the effectiveness of fungicides mixed dry with the soil. Most of the testing has been with the former objective in mind because of the need for an effective soil fungicide that will penetrate soil and kill root pathogens.

Fine sandy loam (pH 7 to 7.5) was air-dried, sifted through a 20-mesh/in. sieve, and autoclaved for 45 minutes at a pressure of 15 lb./sq. in. in 2-qt. Mason jars. For tests of penetration of materials applied in solution or suspension, 1 in. of this soil was placed in a shell vial 25 cc in capacity (20 mm in diameter $\times$ 85 mm deep); a disk 10 mm in diameter was cut from the outer margin of a potato-dextrose-agar culture of the fungus to be tested and placed on the soil (Fig. 1); and the disk was then covered with 1 in. of the steam-sterilized soil. The total volume of the soil in the vial was 17 cc (weight, 20 g). Five ml of the fungicidal solution or suspension to be tested was applied to the surface of the soil in the vial with a pipette or other measuring device; this was sufficient moisture to wet all of the soil in the vial. The vial was then incubated for 24 hours at 25°C. Three replicate vials were used for each concentration of each chemical.

The 3 vials in each test were emptied into a wire or perforated metal strainer, the soil was removed by washing with running water, and the disks of mycelium were picked out with sterile forceps, blotted on paper towels, and placed on agar in Petri dishes to determine viability of the fungus (Fig. 1). In most of the tests, cornmeal agar was used for determination of viability as it is a good medium for growth of the principal test fungus, and as on this agar contaminants do not spread rapidly and interfere with the taking of data on viability of the test fungus.

In tests of fungicides mixed dry with the soil, 60 g of the steam-sterilized soil was placed in a 200-cc Erlenmeyer flask. A measured amount of the dry fungicide was added to the flask, and the fungicide and soil were thoroughly mixed by shaking. One in. of the treated soil was placed in the bottom of each of 3 replicate vials, the inoculum disk was added, the disk covered with 1 in. of the treated soil, and 5 ml of distilled water was applied to the surface of the soil. The vials were then incubated for 24 hours and the disks recovered as noted above. For accurately obtaining extremely low dosages (1–5 ppm), the fungicide was first added to the soil and thoroughly mixed to provide a higher concentration (25 ppm); this mixture was then diluted by adding soil until the desired chemical concentration was reached.

The principal fungus used in these tests was *Phytophthora cinnamomi* Rands. *Verticillium albo-atrum* Reinke & Berth. also was used in a few tests.

Results were recorded merely as presence or absence of growth of the fungus following exposure to the fungicide. The method thus detects primarily the fungicidal materials. Fungistatic chemicals are not determined so readily, although an indication of fungistatic properties may be obtained by comparing the rate of growth of mycelium from the disks from treated soil with that from disks from untreated soil.

In the initial screening tests the fungicides were applied at the rate of 2500 and 500 ppm based on weight of the total amount of soil in the vials. In tests with drenches, 5 ml of a solution or suspension containing 10,000 and 2000 ppm of the chemical was added to 20 g of soil in a vial. Dry materials were mixed with the 20 g of soil at the rates of 50 and 10 mg per vial to give the dosages of 2500 and 500 ppm, respectively. Materials that were fungicidal at the 500-ppm dosage were tested at lower concentrations in the laboratory, and the most effective materials were tested for control of Phytophthora root rot of avocado in the glasshouse. Application of 10 mg of chemical to 20 g of soil is equivalent to applying over 2100 lb. of material per acre-foot of soil.

Incorporation of various concentrations of chemicals in agar at the time of pouring plates is a commonly used and convenient method of testing fungicides. For the agar tests, stock solutions of chemicals were prepared in sterile distilled water. Amounts of chemicals calculated to give the desired series of

concentrations were then pipetted into 125-cc Erlenmeyer flasks containing 45 ml of melted potato-dextrose agar. After thorough mixing of the fungicide, 3 plates were poured from each flask. Disks 5 mm in diameter, cut with a cork borer from cultures of the test fungus on potato-dextrose agar, were placed in the center of each plate. The plates were incubated at 25°C, and growth measurements were taken at intervals. In the results reported, all dosages were calculated on the basis of active ingredient of the fungicide per unit weight of soil or agar.

Forty-seven chemicals, largely organic fungicides, were tested for fungicidal activity in soil. These, together with the active ingredient of each, are listed as follows:[2]

Acti-dione
  3-[2-(3,5-Dimethyl-2-oxocylohexyl)-2-hydroxyethyl] glutarimide
Aluminum derivative of 8-quinolinol
Bioquin 1
  Copper derivative of 8-quinolinol
C & C Experimental Fungicide 1182
  2-(4-Chloro-3,5-xylyloxy)ethanol
Captan
  N-(trichloromethylthio-)4-cyclohexene-1,2-dicarboximide
Ceresan, New Improved (N.I.)
  5% Ethylmercury phosphate
Copper naphthenate
Copper sulfate
Copper and zinc derivative of 8-quinolinol
Crag Potato Fungicide 658
  Copper zinc chromate, $15CuO \cdot 10ZnO \cdot 6CrO_3 \cdot 25H_2O$
Dichlone
  2,3-Dichloro-1,4-naphthoquinone
Dowicide A
  97% Sodium *o*-phenylphenoxide
Dowicide B
  85% Sodium 2,4,5-trichlorophenoxide
Dowicide C
  Sodium chloro-2-phenylphenoxide
Dowicide G
  74% Sodium pentachlorophenoxide (11% sodium salts of other chlorophenols)
Ethyl B-622
  2,4-Dichloro-6(*o*-chloroanilino)-*s*-triazine
Ferbam
  Ferric dimethyldithiocarbamate
8-Hydroxyquinoline sulfate
8-Hydroxyquinoline benzoate
Karathane
  2-(1-Methylheptyl)-4,6-dinitrophenyl crotonate and isomers
Lime sulfur
Maneb
  Manganous ethylenebis[dithiocarbamate]
Mathieson 275
  Pentachloronitrobenzene
Mathieson 1197
  Nitrobenzene
Merculine 100
  10% Phenylmercury salicylate
Nabam
  Disodium ethylenebis[dithiocarbamate]
Natriphene
  Sodium *o*-phenylphenoxide
Pennsalt NP 1083
  1-Fluoro-2,4-dinitrobenzene
Potassium derivative of 8-quinolinol sulfate
Puratized Agricultural Spray
  5% Tris(2-hydroxyethyl) (phenylmercuri) ammonium lactate
Roccal
  10% Mixture of high molecular weight alkyldimethylbenzylammonium chlorides
Rohm & Haas Cr 305
  2,2′-Thiobis[4-chlorophenol]
Rohm & Haas HD-160
  Sodium 2-benzothiazolylmercapto acetate
Rohm & Haas HE-1046
  Sodium dimethyldithiocarbamate
Semesan
  30% 2-Chloro-4-(hydroxymercuri)phenol
Sequestrene AA
  Ethylenediaminetetraacetic acid
Sequestrene Na_2Cu
  Disodium cupric ethylenediaminetetraacetatetrihydrate
Sodium diethyldithiocarbamate
Stauffer N-521
  Tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione
Stauffer N-869 (Vapam)
  Sodium methyldithiocarbamate
Streptomycin
  N-methyl-L-glucosaminidostreptosido-streptidine
Tag 331
  10% Phenylmercury acetate
Thiolutin
  6-Acetamido-4-methyl-1,2-dithiolo[4,3-*b*]pyrrol-5(4*H*)-one.
Thiourea
Thiram
  Bis(dimethylthiocarbamoyl) disulfide
Vancide 51
  Sodium dimethyldithiocarbamate; sodium derivative of 2-mercaptobenzothiazole
Zineb
  Zinc ethylenebis[dithiocarbamate]

[2] Appreciation is expressed to the following manufacturers for supplying chemicals used in these tests: California Spray Chemical Corp.; Carbide and Carbon Chemicals Co.; Dow Chemical Co.; E. I. du Pont de Nemours and Co., Inc.; Ethyl Corp.; Gallowhur Chemical Co.; Geigy Chemical Corp.; Harshaw Chemical Co.; B. L. Lemke and Co., Inc.; Olin Mathieson Chemical Corp.; Monsanto Chemical Co.; Pennsylvania Salt Manufacturing Co., Inc., of Washington; Chas. Pfizer and Co., Inc.; Rohm and Haas Co.; Scientific Oil Compounding Co., Inc.; Stauffer Chemical Co.; United States Rubber Co.; Upjohn Co.; R. T. Vanderbilt Co., Inc.; Winthrop Chemical Co., Inc.; and H. L. Woudhuysen and Associates.

EXPERIMENTAL RESULTS.—The 47 chemicals above were tested for fungicidal activity against *P. cinnamomi* by the drench method in the laboratory. Twenty of these also were tested by the dry-mix method and 25 were assayed for fungicidal activity in agar. Glasshouse trials for penetration of soil to control Phytophthora root rot of avocado seedlings were conducted with 10 of the fungicides. Results obtained by these 4 methods are presented in Table 1.

Fungicide drench tests.—Ten of the 47 materials were fungicidal in the laboratory drench test in glass vials at a dosage of 500 ppm and were tested at lower dosages. The 2 most effective materials in penetrating soil to kill *P. cinnamomi* were Stauffer N-869 and Stauffer N-521. These killed the fungus at a concentration of 5 ppm (Table 1, column A). Nabam and Acti-dione were next in effectiveness, killing *P. cinnamomi* at 25 ppm.

Twenty-five of the 47 chemicals listed above were ineffective in the drench test in vials at 2500 ppm. Eighteen of these were not tested by any of the other methods; results with these materials therefore are not included in Table 1.

Some of these chemicals also were tested against *Verticillium albo-atrum*. Cultures in which microsclerotia were present were used. With several materials, a much higher dosage was required to kill *V. albo-atrum* than to kill *P. cinnamomi* (cultures of the latter fungus did not contain oospores). For example, the lowest dosage at which a nabam drench was fungicidal to *V. albo-atrum* was 250 ppm; whereas nabam was fungicidal to *P. cinnamomi* at 25 ppm. Stauffer N-869 and Acti-dione were fungicidal to *V. albo-atrum* at 50 ppm and 500 ppm, respectively, but were effective against *P. cinnamomi* at 5 ppm and 25 ppm, respectively. Two other materials were fungicidal to both *V. albo-atrum* and *P. cinnamomi* at the following dosages: Pennsalt NP 1083 at 250 ppm and Rohm & Haas HD-160 at 2500 ppm.

To determine actual depth of soil penetration of Semesan, an ineffective fungicide when applied as a

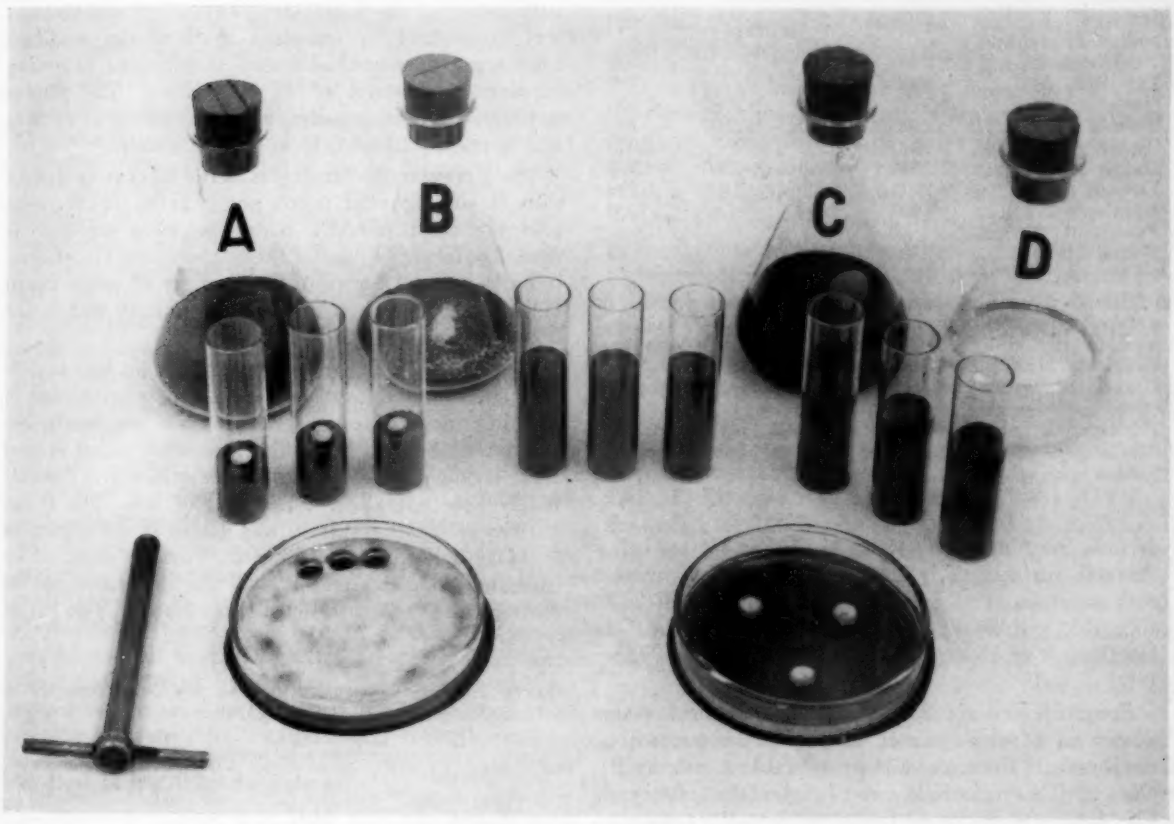


FIG. 1. Laboratory method for testing soil fungicides. For drench tests, inoculum is cut with cork borer from agar plate (left), placed on 1 in. of autoclaved soil from flask A, and covered with 1 in. of autoclaved soil (3 center vials). Five ml of fungicidal solution from flask C is then applied to each vial. Inoculum is recovered after 24-hour incubation, and cultured on agar plate (right) to determine viability. For the dry-mix tests, procedure is similar except that soil and fungicide are thoroughly mixed in flask B; this soil is then used in the vials and 5 ml of water from flask D is applied to each vial.

TABLE 1.—*Lowest concentrations of various chemicals fungicidal to Phytophthora cinnamomi when tested by various methods*

Fungicide	Lowest effective concentration (ppm)[a] when applied			
	A As drench in vials	B As dry mix in vials	C As drench in pots	D In agar
Stauffer N-869 (Vapam)	5	5	12.5	50
Stauffer N-521	5	5	125	50
Nabam	25		1000	40
Acti-dione	25	5		25
Mathieson 1197	50	100		250
Ceresan N.I.	250	5		50
Pennsalt NP 1083	250	50	250	200
Thiolutin	250	50		100
Dowicide G	500	100	500	50
Vancide 51	500			500
Aluminum derivative of 8-quinolinol	2500	>2500[b]		50
Copper sulfate	2500	>2500		1000
8-hydroxyquinoline benzoate	2500	1250		25
8-hydroxyquinoline sulfate	2500	>2500	2500	10
Rohm & Haas HE-1046	2500	1250		1000
Semesan	2500	250		100
Sodium diethyldithiocarbamate	2500			500
Tag 331	2500			10
Zineb	>2500	100	>2500	100
Bioquin 1	>2500	250	>2500	100
Captan	>2500	500		2000
Maneb	>2500	2500	>2500	200
Ferbam	>2500	>2500	>2500	200
Mathieson 275	>2500	>2500		>2500
Puratized Agricultural Spray	>2500			25

[a] Based on active ingredient. The following materials were effective in the drench test in vials at 2500 ppm but were not tested by other methods: C & C Experimental Fungicide 1182, Ethyl B-622, Merculine 100, and Rohm & Haas HD-160. Other materials listed in the text were ineffective as drenches in vials at 2500 ppm.

[b] Materials noted as effective at >2500 ppm were ineffective at 2500 ppm but were not tested at higher concentrations.

drench, inoculum was placed ¼, ½, and 1 in. below the soil surface. Triplicate vials were then treated with suspensions of Semesan to give dosages of 2500, 500, 100, and 50 ppm. Results (Table 2) show an inactivation or absorption of this material in the upper 1 in. of soil.

Fungicide dry-mix tests.—Of the 20 chemicals tested as dry mixes with the soil, 12 killed *P. cinnamomi* at the threshold dosage of 500 ppm (Table 1, column B). Nine of these materials were fungicidal at 100 ppm. The most effective materials were Acti-dione, Ceresan N.I., Stauffer N-869, and Stauffer N-521. These killed *P. cinnamomi* at a dosage of 5 ppm. It should be noted that these results are based on the active ingredient; thus Ceresan N.I., containing 5 per cent ethylmercury phosphate, would be fungicidal at 20 times the above dosage or at 100 ppm.

Chemicals that were appreciably more fungicidal when mixed with the soil than when applied as a drench included Bioquin 1, captan, Semesan, and zineb. These materials are apparently unable to penetrate soil but are fungicidal when incorporated with soil. Results with zineb were somewhat erratic, with indications of a bimodal fungitoxicity curve (9).

Fungicide tests in agar.—Of 25 chemicals tested in potato-dextrose agar, 20 were fungicidal to *P. cinnamomi* at 500 ppm (Table 1, column D). Fourteen of these chemicals prevented growth of the fungus at a dosage of 100 ppm or lower. The most effective fungicides in the agar test were 8-hydroxyquinoline sulfate and Tag 331. It will be noted that there is little correlation between fungicidal value in agar and in the soil tests.

Fungicide tests in the glasshouse.—To determine whether the drench tests in vials containing soil were providing a sound basis for selection of soil fungicides, 10 materials were tested in the glasshouse. Mexican avocado seedlings were transplanted into 6-in. pots containing soil naturally infested with *P. cinnamomi*, and pots were immediately watered with solutions or suspensions of the fungicides. Fungicidal effectiveness was determined by culturing roots of the seedlings after a growth period of several months and recording recovery or absence of *P. cinnamomi*. The fungus produces root rot rapidly under glasshouse conditions and is readily isolated from control plants.

The 2 most active fungicides in the laboratory drench tests in soil, Stauffer N-869 and Stauffer N-521, were also the most effective materials when applied as drenches to pots of soil (Table 1, column C). Correlation was good between effectiveness of other fungicides in the drench tests in the laboratory and in the drench test in the glasshouse.

Influence of soil type.—Fine sandy loam was used in the routine laboratory tests because of convenience in handling and because it is a type commonly encountered. Several fungicides also were tested in clay loam for comparison. Results with nabam and Stauffer N-521 were not affected by types of soil. The fungitoxicity of copper sulfate was found to be higher in the clay soil than in the loam. Further examination indicated that the reason was the difference in the hydrogen-ion concentration of the 2 soils. The pH of

TABLE 2.—*Soil penetration by the fungicide Semesan as indicated by kill of Phytophthora cinnamomi at different depths*

Dosage ppm active ingredient	Inoculum placed in soil at depth of[a]		
	¼ in.	½ in.	1 in.
2500	+	+	+
500	+	+	−
100	+	−	−
50	−	−	−

[a] + = fungus killed; − = no effect.

the fine sandy loam was 7.5; that of the clay loam was 6.5. Acidification of the former soil resulted in increased toxicity of the copper sulfate applied to this soil. This indicates the importance of uniformity in pH and chemical composition of soil used for comparison of fungicides. For a particular disease problem, soil of a pH and type similar to that encountered in the field could be used.

Influence of exposure period.—In the majority of the screening tests the vials of soil were incubated for 24 hours after treatment. Longer periods of incubation were tried with several materials, but an additional 24-, 48-, or 72-hour exposure did not increase the fungicidal action. In 1 test, varying concentrations of nabam and of zineb were applied to the soil, and the vials were incubated for 24, 48, 72, and 96 hours. The concentrations that were ineffective in killing the test fungus after a 24-hour incubation period were also ineffective after a 96-hour incubation period. Conceivably, however, some materials may act more slowly than others. If vapor-phase data or other physical data indicate that such may be the case with a given chemical, a longer period of incubation could be used. For a standard rapid screening test, however, a 24-hour incubation period is convenient and, on the basis of data at hand, sufficient.

Influence of age and amount of inoculum.—It has long been recognized (2, 7) that age of spores has an effect on their susceptibility to toxicants. An effect of age on susceptibility of mycelium to fungicidal action in standard laboratory tests has been reported by Zentmyer and Klotz (10), who found striking differences in the response to chemicals of 1-day-old and 8-day-old mycelia. Standard procedure in the soil-fungicide tests was to take inoculum from the outer actively growing portion of a Petri-dish culture. Differences could be expected if some of the test disks were taken from the center of a culture (mycelium 10–15 days old) and others from the outer margin (mycelium 1–2 days old).

In the routine tests, disks 10 mm in diameter and 2 mm thick were cut from fungus mats growing on potato-dextrose agar. Any aerial mycelium present was included in the inoculum. Trials showed that the inoculum of *P. cinnamomi* from cornmeal agar was more susceptible to fungicides than inoculum from potato-dextrose cultures. Growth of this fungus is more luxuriant on potato-dextrose agar than on cornmeal agar. Thus, a medium that supports vigorous growth of the test fungus should be used in order to avoid conditions that are excessively favorable for action of the toxicants. Tests also showed that a lower dosage is required to kill a fungus when a 5-mm disk of inoculum is used than when a 10-mm disk is used. Both of the factors are related to inoculum potential. In the glass-slide fungicide testing method, Horsfall et al (4) have shown that there is a relation between number of spores in the spore drop and the effective dose of the fungicide, the probit response varying with the log dose per spore. The same relation would be expected to hold true wherever fungicidal action is measured.

Discussion.—On the basis of glasshouse tests, the soil fungicide testing method described in this study is more efficient in selecting chemicals that are effective in soil against root pathogens than are the standard laboratory fungicide testing methods. The new method therefore should prove of value to the investigator who is concerned with such pathogens. The method is simple and involves inexpensive equipment; results are obtained in 24 hours, and large numbers of materials can be screened against a variety of soil organisms in a relatively short period.

The fungitoxicity of many materials that are highly effective in other types of tests was strikingly reduced when they were tested by the soil-drench method. Of the 47 materials tested in this study only 10 were effective at 500 ppm on the basis of weight of active ingredient per unit weight of soil. Fungicidal effectiveness in the drench test is not necessarily directly correlated with water solubility: several of the effective materials are not water soluble, and a number of the ineffective materials, including phenylmercury acetate, Puratized Agricultural Spray, and 8-hydroxyquinoline sulfate, are extremely water soluble.

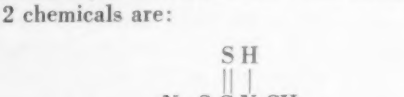
Even though most of the other materials are inherently fungitoxic, either their effectiveness is greatly reduced by adsorption of the active material when the solution or suspension is passed through 1 in. of soil, or they are inactivated on contact with soil. Data presented indicate that both phenomena occur. Some of the materials that were ineffective as drenches were highly effective when mixed with the soil; others had no activity in soil even when mixed with the soil at high dosage. The screening effect of soil on fungitoxicity of Semesan is evident from the data of Table 2.

A considerably higher percentage of fungicides was effective at 500 ppm when mixed with the entire soil volume in the test than when used in the drench applications. Of the 20 materials tested by the soil-mix method, 12 were fungitoxic at 500 ppm and 9 were fungitoxic at 100 ppm. The 12 chemicals included 8 dry-powder formulations that were effective at 500 ppm or less in the drench test, plus 4 chemicals that were ineffective in the drench test. The soil-mix method may be expected to detect materials that would be effective in cases where fungicides can be incorporated into soil or where fungicidal action is needed only at or near the soil surface against postemergence damping-off, crown rots, and some of the turf diseases. These materials might not be detected by the drench screening method, although it is possible that placing inoculum at a depth of ¼ to ½ in. instead of 1 in. would solve this difficulty and simplify the screening method to 1 type of test. The soil-mix method is more time consuming, and unless large volumes of soil are used it is difficult to obtain, with accuracy, dosages much below 5 ppm.

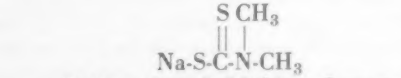
The lack of correlation between fungicidal action in agar and in soil was striking. Five fungicides were effective against *P. cinnamomi* at a dosage of 25 ppm or less in agar: Acti-dione, 8-hydroxyquinoline benzoate, 8-hydroxyquinoline sulfate, Puratized Agricultural Spray, and Tag 331. Of these, only Acti-dione was an effective fungicide in soil.

Phytotoxicity is another important phase of the search for effective soil fungicides, particularly where application of the materials to living plants is involved. Although Stauffer N-869 and N-521 were considerably more fungicidal than nabam, for example, these materials also were more phytotoxic to avocado seedlings than was nabam. Details of tests on phytotoxicity and of fungicidal tests in the glasshouse and field will be presented in a subsequent publication.

Some conclusions may be drawn regarding the relation between structure and fungicidal activity. The most striking examples occur in the dithiocarbamates. Substitution of a single methyl group on the dithiocarbamate nucleus, as in sodium methyldithiocarbamate (Stauffer N-869), results in a highly effective fungicide. This material was approximately 500 times more effective in soil than 2 similar compounds: 1 with 2 methyl groups, sodium dimethyldithiocarbamate (Rohm & Haas HE-1046), and 1 with 2 ethyl groups, sodium diethyldithiocarbamate. The formulae of the first 2 chemicals are:



Sodium methyldithiocarbamate



Sodium dimethyldithiocarbamate

The drench test in soil involves 2 factors: inherent fungitoxicity plus ability to penetrate soil. A more accurate measure of actual fungitoxicity is obtained when the fungicides are tested in agar than by any of the other methods. In tests in agar, sodium methyldithiocarbamate was 20 times as toxic as sodium dimethyldithiocarbamate (Table 1).

In tests with the ethylenebisdithiocarbamates, highly significant differences were noted between the toxicity of the sodium, and the zinc and manganese salts. In the drench test the sodium salt (nabam) was more than 100 times as fungicidal as the zinc salt (zineb) or the manganese salt (maneb). In agar the differences were less striking: nabam was fungicidal at 40 ppm, zineb at 100 ppm, and maneb at 200 ppm (Table 1). Sodium pentachlorophenoxide had appreciable fungicidal action in soil, while sodium 2,4,5-trichlorophenoxides and pentachloronitrobenzene had no activity against the test fungus.

It is a well-known fact that some fungicides are highly specific in their action. Ineffectiveness of a chemical in the tests reported here against *Phytophthora cinnamomi* does not necessarily indicate that this chemical has no possible application as a soil fungicide. Pentachloronitrobenzene, for example, had no fungicidal value in these tests, but it is known to be effective in soil against several other soil pathogens (1). Kendrick and Middleton (5) showed this material to be highly specific in its action in agar tests. For this reason, in screening chemicals for general use as soil fungicides, several different types of test organisms should be used.

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PHYTOPATHOLOGICAL NOTES

Longevity of the Oak Wilt Fungus Stored Under Mineral Oil. CHARLES L. FERGUS AND HERBERT COLE, JR.[1] During the course of research on oak wilt, hundreds of isolates of *Endoconidiophora fagacearum* Bretz are obtained which cannot be investigated immediately or be examined critically because of limitations on time and materials or because of other duties and activities. The retention of these isolates for prolonged periods involves repeated transfer and therefore increases the possibility of alteration of various characteristics, including pathogenicity. Furthermore, their maintenance by ordinary culture methods on agar media for any length of time requires a considerable amount of work.

A possible solution to these problems lies in the use of sterile mineral oil to cover the stored cultures. This method of preservation of pure cultures of bacteria[2], filamentous fungi[3, 4], and yeasts[5] has been known for some time. Wernham and Miller[6] have shown that *Diplodia zeae*, *Gibberella zeae*, and *Helminthosporium maydis* retained their pathogenicity for 3 years under oil. After 2 years the pathogenicity of *Phytophthora infestans* was slightly reduced. The following study was conducted to determine if *E. fagacearum* could be stored under oil for prolonged periods without the loss of desired characteristics.

Fresh transfers of 15 isolates on test tube slants of malt extract agar and potato dextrose agar were allowed to grow at 25°C for 14 days. The cultures were then covered with a layer of sterile mineral oil and stored at 12°C. At yearly intervals for 3 years, viability was tested by removing a piece of the mycelial colony with a sterile transfer needle, allowing the excess oil to drain off, and then planting the mycelium in malt extract agar in petri dishes. In all of the transfers, *E. fagacearum* grew away from the transferred mycelium and formed its characteristic growth pattern. Also, conidiophores and endoconidia were produced in abundance indicating that it had remained viable for 3 years under mineral oil.

Transplants of the colonies of 2 isolates from the third-year sampling were used to determine pathogenicity. Two-year-old red oak seedlings were wounded by making slanting cuts with a sterile scalpel through the bark and just into the outer xylem. The cuts were made 6 in. above the soil level. The bark was then peeled back for ½ in. and a spore suspension brushed onto the wounds with a sterile brush. The spore suspension had been adjusted to 1 million spores per ml and a viability test showed 85 per cent germination. A small amount of moistened sterile absorbent cotton was wrapped around the wound. The seedlings were kept in insect-proof cages in the greenhouse. Typical foliage symptoms of oak wilt appeared on the inoculated seedlings after 45 days. *E. fagacearum* was successfully reisolated from the wilting seedlings.

The cultures were spermatized successfully with endoconidia of the appropriate compatibility type. Numerous typical perithecia and ascospores were produced.

These experiments demonstrate that the mineral oil preservation method may be used with cultures of *E. fagacearum* for a period of at least 3 years with little or no alteration of the morphological, sexual, and pathogenic characteristics of the organism. The method provides for prolonged storage of the organism without frequent transfers or critical examination.—Department of Botany and Plant Pathology, Pennsylvania State University, State College Pennsylvania.

[1] Associate Professor and Research Assistant, respectively. Contribution No. 192 from the Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication December 8, 1954, as paper No. 1930 in the Journal Series.

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[4] Buell, C. B., and W. H. Weston. 1947. Application of the mineral oil conservation method to maintaining collections of fungus cultures. Amer. Jour. Bot. 34: 555-561.

[5] Henry, B. S. 1947. The viability of yeast cultures preserved under mineral oil. Jour. Bact. 54: 264.

[6] Wernham, C. C., and H. J. Miller. 1948. Longevity of fungus cultures under mineral oil. Phytopathology 38: 932-934.

A Simple Device for Single Spore Isolation.[1] CHARLES M. LEACH. The isolator described is of the "biscuit cutter" type and is so mounted on the microscope objective that the cutter remains in the field of view continuously during the isolation of the spore. The device incorporates some features of isolators designed by Keitt,[2] LaRue,[3] Sass,[4] and Lambert[5] and can be constructed inexpensively. The isolator consists of the following parts: 1) A holder fitted snugly over a 16-mm microscope objective and held against

[1] Approved for publication as Technical Paper No. 889 by Director of the Oregon Agricultural Experiment Station. Photographs were made by H. H. Millsap, Oregon Agricultural Experiment Station, Corvallis, Oregon.

[2] Keitt, G. W. 1915. Simple technique for isolating single-spore strains of certain types of fungi. Phytopathology 5: 266-269.

[3] Lambert, E. B. 1939. A spore isolator combining some of the advantages of the La Rue and Keitt methods. Phytopathology 29: 212-214.

[4] LaRue, C. D. 1920. Isolating single spores. Bot. Gaz. 70: 319-320.

[5] Sass, J. E. 1929. The cytological basis for homothallism and heterothallism in the Agaricaceae. Amer. Jour. Bot. 16: 663-701.

the base of the objective by a small thumb screw (Fig. 1, 2). The holder may be made from a small piece of aluminum or brass approximately 7 mm thick. 2) A cutting device fashioned from a 4-cm portion of a bicycle spoke bent as shown in Figure 3. One end of the spoke was flattened with a hammer and shaped into a cylindrical cutter having a diameter of 1.5 mm. The needle was held in the holder by means of a thumb screw to permit adjustment of the vertical and radial position of the cutter. 3) A handle permanently fixed to the holder to facilitate removal for flaming. The isolator can be used as follows: 1) Convenient containers are filled to a depth of at least 3 mm with 4 per cent clear agar. When agar of less than 4 per cent is used moisture may be exuded by the pressure of the cutter and the spore may float out of position. The spores are spread over the surface of the agar by any of the commonly used methods. 2) Before isolations are made, the isolator is slipped onto the objective and fastened against the base of the objective with the thumb screw. The cutter is then adjusted in the field of view until it appears slightly above the focal plane (Fig. 4, A). 3) The isolator is removed from the objective and the agar surface is scanned for a well-placed spore (Fig. 4, B). 4) The isolator is flamed and replaced on the objective where the cutter automatically lines up in the center of the field of view. 5) The objective is focused until the single spore comes into view through the center of the slightly out-of-focus cutter (Fig. 4, C), then racked down until the cutter penetrates the agar to at least twice its depth (Fig. 4, D). 6) On raising the objec-

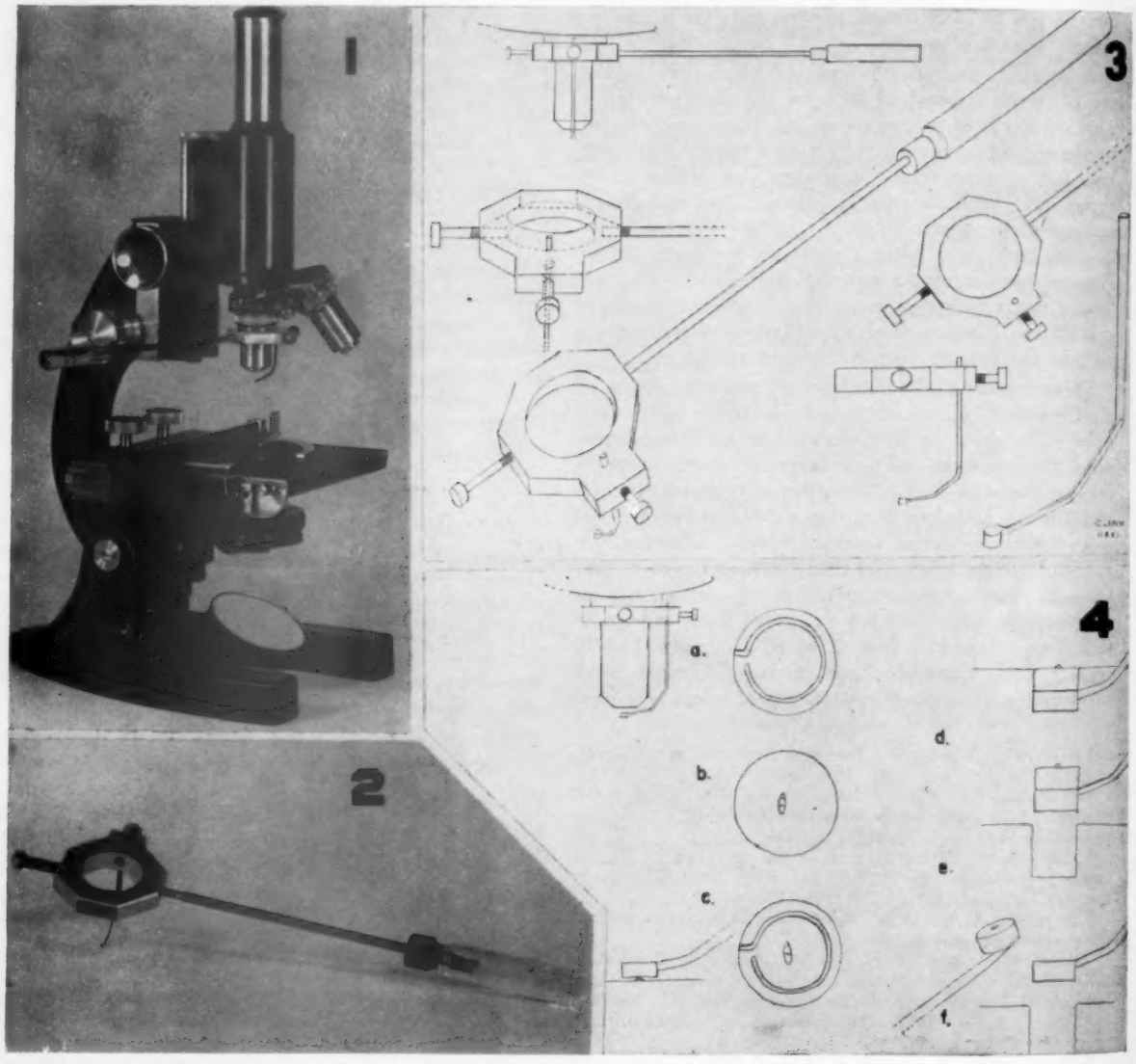

FIG. 1–4. Fig. 1. Single-spore isolator in position on 16-mm microscope objective.—Fig. 2. Isolator detached from objective.—Fig. 3. Drawings of isolator (not to scale).—Fig. 4. Single-spore isolation procedure.

tive, a minute agar-plug is removed (Fig. 4, E). Part of this plug, with the single spore on its surface, protrudes above the top of the cutter. The protruding agar and spore can be readily removed for transplanting (Fig. 4, F). 7) Further isolations may be performed quickly by removing the isolator from the objective, flaming the cutter, replacing the isolator, and repeating the above procedure.

The advantages of this mechanism are: 1) since the cutter is continuously in the field of view, it can be observed as it penetrates the agar, and 2) the isolator can be removed easily and quickly for flaming, and replaced in exactly the same position without removing the objective from the microscope.—Department of Botany and Plant Pathology, Oregon Agricultural Experiment Station, Corvallis.

The Production and Role of Antibiotics in Soil. V. Antibacterial Activity of Five Antibiotics in the Presence of Soil. MARY MARTIN AND DAVID GOTTLIEB. That the production of antibiotics in soil can influence the severity of disease caused by plant pathogenic soil fungi and bacteria has been frequently suggested. Acceptance of this hypothesis is contingent upon 2 factors, 1) production of antibotics in soil and 2) accumulation of such materials in inhibitory concentration. Previous publications have presented data on this subject.[1,2,3,4] The current report is a continuation of these studies with 5 other substances, circulin, viomycin, subtilin, actinomycin, and neomycin. All of these antibiotics, except neomycin, have 1 feature in common, they contain amino acids in peptide linkages as part of the molecule. Information of 2 kinds was sought: first, whether soil would remove the antibiotic from solution and, second, whether the presence of the antibiotic in soil would inhibit the growth of certain soil-inhabiting bacteria.

Many of the techniques used were those that have been reported previously. The sensitivities of the test organisms to the different materials were determined by the test-tube dilution procedure.[5] Soil inactivation studies were usually made by placing 1.0 g of a sandy loam soil and 5 ml of nutrient broth in test tubes and then sterilizing the suspension in an autoclave. Solutions of antibiotic at a concentration of 1000 μg per ml were sterilized by sintered glass filters, and aliquots were added to the tubes containing the sterile broth-soil suspensions. These mixtures were shaken on a gump rotary shaker for 2 hours to allow for reaction between the soil and the antibiotic and then centrifuged so that a clear layer of broth remained above the compacted soil. One drop of a bacterial suspension was added to the supernatant liquid and the test system was incubated for 18 hours at 30°C. The activity of the antibiotic under these conditions was determined by measuring the growth of the bacteria turbidimetrically. The growth of the bacteria in the complete system was compared to that in the control tubes to which no antibiotic had been added and in which the bacteria grew prolifically. Other controls consisted of tubes containing the same concentration of antibiotic used in the test series. A slight modification was used for testing the activity of actinomycin. This compound was dissolved directly in nutrient broth and sterilized by filtration. The tubed soil was sterilized separately and the nutrient media containing actinomycin at different concentrations were then added. All broth experiments were in duplicate, whereas experiments involving bacteria in soil were run in triplicate.

Bacilus subtilis Cohn (Pr.) was used for testing the inhibitory effect of 4 of the antibiotics. These inhibited growth in liquid media at the following concentration in μg. per ml: circulin, 5.0; neomycin, 0.4; viomycin, 20.0; and actinomycin, 0.1. *Bacillus cereus* Frankl. & Frankl., on the other hand, was used with subtilin and was inhibited at a level of 20.0 μg per ml.

In the presence of soil most of the antibiotics were ineffective in preventing the growth of the bacteria. Even concentrations of 500 μg per g of soil did not inhibit the growth of *B. subtilis* when circulin, neomycin, or viomycin were used, nor of *B. cereus* in the presence of similar concentrations of subtilin. In contrast to these materials, actinomycin was very active in the presence of soil. Bacterial growth took place normally at 1.0 μg of antibiotic per g of soil but decreased gradually as the concentration was increased to 5 μg; complete inhibition occurred at 6 μg per g. Since *B. subtilis* was inhibited by 0.1 μg per ml in the absence of soil, it is obvious that the soil prevented the activity of a large proportion of the original actinomycin.

In order to determine the degree of adsorption of actinomycin, nutrient broth containing this compound was mixed with soil so that a concentration of 500 μg per g was obtained. Biological "paper-disc" assays[5] of the nutrient broth both before and after it had been mixed with soil revealed only 50 μg of actinomycin per g of soil remaining in solutions after mixing. This represents an adsorption of 90 per cent.

The population of the different bacteria in soil to which the antibiotics had been added is given in Table 1. These results confirm those obtained from the soil-broth experiments. Even at concentrations

[1] Gottlieb, D., and P. Siminoff. 1952. The production and role of antibiotics in the soil. II. Chloromycetin. Phytopathology 42: 91-97.

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[3] Martin, Norma, and D. Gottlieb. 1952. The production and role of antibiotics in the soil. III. Terramycin and aureomycin. Phytopathology 42: 294-296.

[4] Siminoff, P., and D. Gottlieb. 1951. The production and role of antibiotics in the soil. I. The fate of streptomycin. Phytopathology 41: 420-430.

[5] Florey H. W., E. Chain, N. G. Heatley, M. A. Jennings, A. G. Sanders, E. P. Abraham, and M. E. Florey. 1949. Antibiotics. Oxford University Press, New York. 2 v. 1774 p.

of 500 μg per g there was no diminution in the number of bacteria compared to the controls for any of the materials except actinomycin. This antibiotic inhibited growth to 2.7 per cent of that which occurred in its absence. An aqueous suspension of actinomycin has a pH of 7.2 indicating that it is a weak base and thus would be adsorbed by soil that has an hydrogen ion concentration on the acid side. The pH of the soils differed at the end of the experiment, depending on the antibiotic that had been added (Table 1).

TABLE 1.—*Bacterial population after 9 days of growth in soil containing antibiotics*

Antibiotic	Concentration	Number of bacteria per g of soil		Final pH of soil
		B. subtilis	*B. cereus*	
	μg/g soil	$\times 10^5$	$\times 10^5$	
Control	500	148	39	6.0
Subtilin	500		49	6.1
Circulin	500	177		6.3
Viomycin	500	199		6.3
Neomycin	500	148		6.3
Actinomycin	500	4		5.7

The antibiotics subtilin, circulin, viomycin, and neomycin, did not inhibit the growth of sensitive bacteria in soil that had been previously sterilized. Actinomycin, on the other hand, inhibited growth at relatively low concentrations. All of these antibiotics apparently were removed to some extent from solution by soil. The antibiotic activity of actinomycin in soil despite its adsorption might be explained by the very high activity of this compound against the test organism. Since only 0.1 μg per ml is necessary to inhibit *B. subtilis* in broth, and 6 μg per g are necessary in the presence of soil, inhibition would have occurred even though 98 per cent of the compound was inactivated by the soil. If the same percentage adsorption took place when the antibiotic was added directly to soil at a concentration of 500 μg per g, 10 μg would remain free in the soil solution—more than enough to prevent the growth of *B. subtilis*. It is apparent that of the 5 antibiotics studied, only actinomycin might inhibit plant pathogens in the silt loam soil of central Illinois.—Department of Horticulture, University of Illinois, Urbana, Illinois.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

McCubbin, W. A. 1954. **The plant quarantine problem.** Ejnar Munksgaard, Copenhagen. (American distributors: The Chronica Botanica Co., Waltham 54, Mass.) 255 p. Price $4.80.

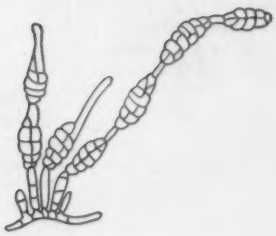
This book is an over-all review of the subject of plant quarantines—their biological background, purposes, methods, procedures, limitations, and possibilities. Consideration is given to social and economic relations, and to legal and administrative aspects. Although the book is concerned primarily with the United States situation, the world situation is discussed in the final chapter. The appendix consists of a historical summary of Federal plant disease quarantines.

Day, W. R. 1955. **Forest Hygiene in Great Britain.** University of Toronto Forestry Bulletin No. 4, Univ. of Toronto Press, Toronto, Canada. 26 p. 6 plates. Price $1.00.

The author, Lecturer in Forest Pathology and Forest Hygiene at the University of Oxford, here presents a summary of his researches and observations on problems having to do with the health of the forest and more particularly with the relation of environmental factors to the establishment and development of young stands. The material contained in this volume was presented in a series of lectures to the School of Graduate Studies, University of Toronto, at the invitation of the Department of Botany and Forestry. There is a bibliography of 52 titles.

DIFCO

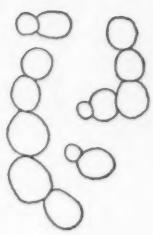
Media for



MYCOLOGY

DIAGNOSTIC

. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:



Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

. . . and nutritional studies of fungi:

Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION,*
including descriptions of these media and their use,
is available on request.

DIFCO LABORATORIES
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A testimonial dinner honoring W. D. Valleau was held April 30 at the University of Kentucky. The dinner was attended by more than 400 people from Kentucky and elsewhere representing farmers, businessmen, bankers, burly tobacco warehousemen, tobacco manufacturers, seed growers, and research workers. As an acknowledgement of his many outstanding contributions over the past 35 years to agriculture and to the production of burley tobacco through his disease control and breeding studies, Dr. Valleau was presented with a Cadillac automobile.

Visiting scientist Mary T. Franklin of the Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts, England, is spending the months of July, August, and September at the Connecticut Agricultural Experiment Station, New Haven. Dr. Franklin will conduct research and will act as a nematology consultant while at New Haven.

Phytopathological Classics Nos. 1 and 2 have been reprinted and are available now at $0.75 each from Carl W. Boothroyd, Department of Plant Pathology, Cornell University, Ithaca, New York.

Phytopathologist W. J. Zaumeyer has received a grant from the Rockefeller Foundation and will spend a month in Colombia and Mexico to confer with Dr. R. L. Skiles and Dr. W. D. Yerkes and other staff members on the Bean Improvement Program carried on by The Foundation in those countries. While in Colombia, Dr. Zaumeyer will also attend the Third Latin-American Reunion of Geneticists, Pathologists, Entomologists and Soil Scientists, which is being held at Bogota, Colombia from June 20 to July 1, 1955.

Congratulations to pathologist Harold T. Cook who was appointed Head of the Quality Maintenance and Improvement Section of the Marketing Research Division in the U.S.D.A.

The North Central Division will hold its summer meeting August 8-10 at Wooster, Ohio. Registration will be at 10:00 a.m. on August 8 followed by an inspection of exhibits. A symposium on "Systemics" will be held the evening of August 8. On August 9, field plots will be inspected and a joint banquet and program will be held with the Ohio Pesticide Institute during the evening. Visits to outlying experiments will be made August 10. Further details concerning the program can be obtained by contacting H. C. Young, Department of Botany, Ohio Agricultural Experiment Station, Wooster, Ohio.

Deaths: Walter A. McCubbin, associated for 30 years with the U.S.D.A. Bureau of Entomology and Plant Quarantine, died June 13 at the age of 75. Dr. McCubbin headed the field laboratory of plant pathology at St. Catherine's, Ontario, in 1912 and in 1919 was appointed assistant director of the Pennsylvania State Bureau of Plant Industry.

C. R. Orton retired Dean of the West Virginia University College of Agriculture died June 16 at the age of 70. Dr. Orton was a former president of the Society and was internationally known for his work on taxonomy of the Uredinales and Dothideales as well as for his investigations on potato diseases.

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

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